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(54) Title: PORPHYROMONAS GINGIVALIS ARGININE-SPECIFIC PROTEINASE CODING SEQUENCES			
(57) Abstract Provided herein is a nucleotide sequence encoding an Arg-specific gingipain named gingipain-1 isolated from Porphyromonas gingivalis, having an apparent molecular mass of 50 kDa as estimated by SDS gel electrophoresis and an apparent molecular mass of 44 kDa as estimated by gel filtration chromatography. Gingipain-1 has amidolytic and proteolytic activity for cleavage after arginine residues and has no amidolytic and/or proteolytic activity for cleavage after lysine residues. Its activity is inhibited by cysteine protease group-specific inhibitors and chelating agents. It is stabilized by Calcium and stimulated by glycine-containing peptides and glycine analogues.			

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PORPHYROMONAS GINGIVALIS ARGININE-SPECIFIC PROTEINASE
CODING SEQUENCES

This invention was made, at least in part, with funding from
5 the National Institutes of Health (Grant Nos. DE 09761, HL 26148
and HL 37090). Accordingly, the United States Government may
have certain rights in this invention.

FIELD OF THE INVENTION

10 The field of this invention is bacterial proteases, more
particularly those of Porphyromonas gingivalis, most particularly
the arginine-specific protease termed Arg-gingipain herein and
the nucleotide sequences encoding same.

15 BACKGROUND OF THE INVENTION

Porphyromonas gingivalis (formerly Bacteroides gingivalis)
is an obligately anaerobic bacterium which is implicated in
20 periodontal disease. P. gingivalis produces proteolytic enzymes
in relatively large quantities; these proteinases are recognized
as important virulence factors. A number of physiologically
significant proteins, including collagen, fibronectin,
immunoglobulins, complement factors C3, C4, C5, and B, lysozyme,
25 iron-binding proteins, plasma proteinase inhibitors, fibrin and
fibrinogen, and key factors of the plasma coagulation cascade
system, are hydrolyzed by proteinases from this microorganism.
Such broad proteolytic activity may play a major role in the
evasion of host defense mechanisms and the destruction of
30 gingival connective tissue associated with progressive
periodontitis (Saglie et al. (1988) *J. Periodontol.* 59, 259-265).

There are conflicting data as to the number and types of proteinases produced by P. gingivalis. In the past, proteolytic activities of P. gingivalis were classified into two groups; those enzymes which specifically degraded collagen and the general "trypsin-like" proteinases which appeared to be responsible for other proteolytic activity. Trypsin (and trypsin-like proteases) cleaves after arginine or lysine in the substrates (See, e.g. Lehninger A. L. (1982), Principles of Biochemistry, Worth Publishing, Inc., New York). Although many attempts have been made to separate one of these trypsin-like proteinases, Chen et al. (1992) *J. Biol. Chem.* 267, 18896-18901 reported the first rigorous purification and biochemical and enzymological characterization for an Arginine-specific P. gingivalis protease.

This application reports the purification of 50 kDa and high molecular weight trypsin-like, thiol-activated proteinases of P. gingivalis and nucleotide sequences encoding same.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a nucleotide sequence encoding a low molecular weight Arg-gingipain, termed Arg-gingipain-1 (or gingipain-1), herein, said gingipain-1 having an apparent molecular mass of 50 kDa as estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and an apparent molecular mass of 44 kDa as estimated by gel filtration chromatography, said gingipain-1 having amidolytic and proteolytic activity for cleavage after arginine residues and having no amidolytic and/or proteolytic activity for cleavage after lysine residues, wherein the amidolytic and/or proteolytic activity is inhibited by cysteine protease group-specific inhibitors including iodoacetamide, iodoacetic acid, N-ethylmaleimide, leupeptin, antipain, trans-epoxysuccinyl-L-leucylamido-(4-guanidine)butane, TLCK, TPCK, p-aminobenzamidine, N-chlorosuccinamide, and chelating agents including EDTA and EGTA, wherein the amidolytic and/or

proteolytic activity of said gingipain-1 is not sensitive to inhibition by human cystatin C, α 2-macroglobulin, α 1-proteinase inhibitor, antithrombin III, α 2-antiplasmin, serine protease group-specific inhibitors including diisopropylfluorophosphate, phenylmethyl sulfonylfluoride and 3,4-diisochlorocoumarin, and wherein the amidolytic and/or proteolytic activities of gingipain-1 are stabilized by Ca^{2+} and wherein the amidolytic and/or proteolytic activities of said gingipain-1 are stimulated by glycine-containing peptides and glycine analogues. In a specifically exemplified gingipain-1 protein, the protein is characterized by an N-terminal amino acid sequence as given in SEQ ID NO:1 Tyr-Thr-Pro-Val-Glu-Glu-Lys-Gln-Asn-Gly-Arg-Met-Ile-Val-Ile-Val-Ala-Lys-Lys-Tyr-Glu-Gly-Asp-Ile-Lys-Asp-Phe-Val-Asp-Trp-Lys-Asn-Gln-Arg-Gly-Leu-Thr-Lys-Xaa-Val-Lys-Xaa-Ala) and by a C-terminal amino acid sequence as given in SEQ ID NO:6 (Glu-Leu-Leu-Arg).

A further object of this invention is a nucleotide sequence encoding a high molecular weight form of Arg-gingipain, termed Arg-gingipain-2 herein, which comprises a proteolytic component essentially as described hereinabove and at least one hemagglutinin component.

As specifically exemplified, the encoded Arg-gingipain-hemagglutinin complex is transcribed as a prepolyprotein, with the amino acid sequence as given in SEQ ID NO:11 from amino acid 1-1704. The encoded mature high molecular weight Arg-gingipain protein has a protease component having a complete deduced amino acid sequence as given in SEQ ID NO:11 from amino acid 228 through amino acid 719. An alternative protease component amino acid sequence is given in SEQ ID NO:4, amino acids 1-510. Arg-gingipain-2 further comprises at least one hemagglutinin component. The hemagglutinin components which are found associated with the 50 kDa Arg-specific proteolytic component are 44 kDa, 27 kDa and 17 kDa, and have amino acid sequences as given in SEQ ID NO:11, from 720 to 1091, from 1092 to 1429 and from 1430 to 1704, respectively.

It is an additional object of the invention to provide nucleic acid molecules for the recombinant production of an Arg-gingipain. Substantially pure recombinant Arg-gingipain-1 protein can be prepared after expression of the nucleotide sequences encoding Arg-gingipain in a heterologous host cell using the methods disclosed herein. Said substantially pure Arg-gingipain-1 exhibits amidolytic and/or proteolytic activity with specificity for cleavage after arginine, but exhibits no amidolytic and/or proteolytic activity with specificity for cleavage after lysine residues. The purification method exemplified herein comprises the steps of precipitating extracellular protein from cell-free culture supernatant of Porphyromonas gingivalis with ammonium sulfate (90% w/v saturation), fractionating the precipitated proteins by gel filtration, further fractionating by anion exchange chromatography those proteins in the fractions from gel filtration with the highest specific activity for amidolytic activity as measured with Benzoyl-L-arginyl-p-nitroanilide and collecting those proteins which were not bound to the anion exchange column, and fractionating those proteins by FPLC over a cation exchange column (MonoS HR5/5, Pharmacia, Piscataway, NJ) and finally separating gingipain-1 from lysine-specific proteolytic/amidolytic protein(s) by affinity chromatography over L-arginyl-agarose. Preferably the P. gingivalis used is strain H66, and preferably the culture is grown to early stationary phase. Arg-gingipain-1 can also be purified from cells using appropriate modifications of the foregoing procedures (cells must be disrupted, e.g., by lysis in a French pressure cell). Preferably the gel filtration step is carried out using Sephadex G-150, the anion exchange chromatography step is carried out using diethylaminoethyl (DEAE)-cellulose, the FPLC step is carried out using Mono S, and the affinity chromatography is carried out using L-arginyl-Sepharose 4B.

It is a further object of this invention to provide recombinant polynucleotides (e.g., a recombinant DNA molecule) comprising a nucleotide sequence encoding an Arg-gingipain

protein, preferably having an amino acid sequence as given in SEQ ID NO:11 from amino acid 228 through amino acid 719 or having an amino acid sequence as given in SEQ ID NO:4, amino acids 1 through 510. As specifically exemplified herein, the nucleotide sequence encoding a mature Arg-gingipain protease is given in SEQ ID NO:10, nucleotides 1630 through 3105, or SEQ ID NO:3 from nucleotides 1630 through 3105. The skilled artisan will understand that the amino acid sequence of the exemplified gingipain protein can be used to identify and isolate additional, nonexemplified nucleotide sequences which will encode a functional protein of the same amino acid sequence as given in SEQ ID NO:4 from amino acid 1 through amino acid 510 or an amino acid sequence of greater than 90% identity and having equivalent biological activity. The skilled artisan understands that it may be desirable to express the Arg-gingipain as a secreted protein; if so, he knows how to modify the exemplified coding sequence for the "mature" gingipain-2 by adding a nucleotide sequence encoding a signal peptide appropriate to the host in which the sequence is expressed. When it is desired that the sequence encoding an Arg-gingipain protein be expressed, then the skilled artisan will operably link transcription and translational control regulatory sequences to the coding sequences, with the choice of the regulatory sequences being determined by the host in which the coding sequence is to be expressed. With respect to a recombinant DNA molecule carrying an Arg-gingipain coding sequence, the skilled artisan will choose a vector (such as a plasmid or a viral vector) which can be introduced into and which can replicate in the host cell. The host cell can be a bacterium, preferably Escherichia coli, or a yeast or mammalian cell.

Also provided is a specific exemplification of a nucleotide sequence encoding an Arg-gingipain, including low molecular weight gingipain-1 protease component and the protease component of high molecular weight gingipain and its associated hemagglutinin components. These components are processed from a prepolyprotein. As specifically exemplified, the coding

sequence, from nucleotide 949 to nucleotide 6063 in SEQ ID NO:10, including the stop codon, encodes a prepolyprotein having an amino acid sequence as given in SEQ ID NO:11. The prepolyprotein is encoded by a nucleotide sequence as given in SEQ ID NO:10 from
5 nucleotide 949 to 6063. The mature protease molecule is encoded at nucleotides 1630 through 3105 in SEQ ID NO:10. The mature Arg-specific proteolytic component has an amino acid sequence as given in SEQ ID NO:11 from 228-719, and the hemagglutinin component has an amino acid sequence as in SEQ ID NO:11 from 720-1091, from
10 1092 to 1429 or from 1430 to 1704.

In another embodiment, recombinant polynucleotides which encode an Arg-gingipain, including, e.g., protein fusions or deletions, as well as expression systems are provided.
15 Expression systems are defined as polynucleotides which, when transformed into an appropriate host cell, can express a proteinase. The recombinant polynucleotides possess a nucleotide sequence which is substantially similar to a natural Arg-gingipain-encoding polynucleotide or a fragment thereof.

20 The polynucleotides include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or contain non-natural or derivatized nucleotide bases. DNA is
25 preferred. Recombinant polynucleotides comprising sequences otherwise not naturally occurring are also provided by this invention, as are alterations of a wild type proteinase sequence, including but not limited to deletion, insertion, substitution of one or more nucleotides or by fusion to other polynucleotide
30 sequences.

The present invention also provides for fusion polypeptides comprising an Arg-gingipain. Homologous polypeptides may be fusions between two or more proteinase sequences or between the
35 sequences of a proteinase and a related protein. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the proteins from

which they are derived. Fusion partners include but are not limited to immunoglobulins, ubiquitin bacterial β -galactosidase, trpE, protein A, β -lactamase, alpha amylase, alcohol dehydrogenase and yeast alpha mating factor, (Godowski et al. (1988) *Science*, 241, 812-816). Fusion proteins will typically be made by recombinant methods but may be chemically synthesized.

Compositions and immunogenic preparations including but not limited to vaccines, comprising recombinant Arg-gingipain derived from P. gingivalis and a suitable carrier therefor are provided. Such vaccines are useful, for example, in immunizing an animal, including humans, against inflammatory response and tissue damage caused by P. gingivalis in periodontal disease. The vaccine preparations comprise an immunogenic amount of a proteinase or an immunogenic fragment or subunit thereof. Such vaccines may comprise one or more Arg-gingipain proteinases, or an Arg-gingipain in combination with another protein or other immunogen. By "immunogenic amount" is meant an amount capable of eliciting the production of antibodies directed against one or more Arg-gingipains in an individual to which the vaccine has been administered.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the composite physical map of an Arg-gingipain locus. The first codon of the mature Arg-gingipain proteolytic component is indicated. Only major restriction sites employed in cloning are indicated: B, *Bam*HI; P, *Pst*I; S, *Sma*I; A, Asp 718; Pv, *Pvu*II; H, *Hind*III. The four arginine cleavage sites (R227, R719, R1091 and R1429) are each indicated with an asterisk (*). The three residues forming the active site (C412, H438 and N669, respectively) are also shown.

Figure 2 is a protein matrix plot, which presents analysis of regions of similarity between hemagglutinin domains using Pustell Protein Matrix from MacVector, Release 4.0. The complete prepolyprotein sequence (SEQ ID NO:11) was used as X-axis and Y-

axis. The perfect diagonal row is the line of identity, whereas structure in the pattern near that diagonal corresponds to internal repeats. The four different domains are represented (Arg-gingipain protease, 44 kDa hemagglutinin, 17 kDa hemagglutinin and 27 kDa hemagglutinin). Four regions of high homology are identified. The main homologies between hemagglutinin domains is shown in detail in Table 4.

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations used herein for amino acids are standard in the art: X or Xaa represents an amino acid residue that has not yet been identified but may be any amino acid residue including but not limited to phosphorylated tyrosine, threonine or serine, as well as cysteine or a glycosylated amino acid residue. The abbreviations for amino acid residues as used herein are as follows: A, Ala, alanine; V, Val, valine; L, Leu, leucine; I, Ile, isoleucine; P, Pro, proline; F, Phe, phenylalanine; W, Trp, tryptophan; M, Met, methionine; G, Gly, glycine; S, Ser, serine; T, Thr, threonine; C, Cys, cysteine; Y, Tyr, tyrosine; N, Asn, asparagine; Q, Gln, glutamine; D, Asp, aspartic acid; E, Glu, glutamic acid; K, Lys, lysine; R, Arg, arginine; and H, His, histidine. Other abbreviations used herein include Bz, benzoyl; Cbz, carboxybenzoyl; pNA, p-nitroanilide; MeO, methoxy; Suc, succinyl; OR, ornithyl; Pip, pipicolyl; SDS, sodium dodecyl sulfate; TLCK, tosyl-L-lysine chloromethyl ketone; TPCK, tosyl-L-phenylalanine chloromethyl ketone; S-2238, D-Phe-Pip-Arg-pNA, S-2222, Bz-Ile-Glu-(γ -OR)-Gly-pNA; S-2288, D-Ile-Pro-Arg-pNA; S-2251, D-Val-Leu-Lys-pNA; Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-propane-1,3-diol; FPLC, fast protein liquid chromatography; HPLC, high performance liquid chromatography; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; EGTA, [ethylene-bis(oxyethylene-nitrile)tetraacetic acid; EDTA, ethylenediamine-tetraacetic acid; Z-L-Lys-pNa, Z-L-Lysine-p-Nitroanilide; HMW, high molecular weight.

Arg-gingipain is the term given to a P. gingivalis enzyme with specificity for proteolytic and/or amidolytic activity for cleavage of an amide bond, in which L-arginine contributes the carboxyl group. The Arg-gingipains described herein have identifying characteristics of cysteine dependence, inhibition response as described, Ca^{2+} - stabilization and glycine stimulation. Particular forms of Arg-gingipain are distinguished by their apparent molecular masses of the mature proteins (as measured without boiling before SDS-PAGE). Arg-gingipains of the present invention have no amidolytic or proteolytic activity for amide bonds in which L-lysine contributes the $-\text{COOH}$ moiety.

Arg-gingipain-1 is the name given herein to a protein characterized as having a molecular mass of 50 kDa as measured by SDS-PAGE and 44 kDa as measured by gel filtration over Sephadex G-150, having amidolytic and/or proteolytic activity for substrates having L-Arg in the P_1 position, i.e. on the N-terminal side of the peptide bond to be hydrolyzed but having no activity against corresponding lysine-containing substrates being dependent on cysteine (or other thiol groups for full activity), having sensitivity to cysteine protease group-specific inhibitors including iodoacetamide, iodoacetic acid, and N-methylmaleimide, leupeptin, antipain, trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane, TLCK, TPCK, p-aminobenzamidine, N-chlorosuccinamide, and chelating agents including EDTA and EGTA, but being resistant to inhibition by human cystatin C, $\alpha 2$ -macroglobulin, $\alpha 1$ -proteinase inhibitor, antithrombin III, $\alpha 2$ -antiplasmin, serine protease group-specific inhibitors including diisopropylfluorophosphate, phenylmethyl sulfonylfluoride and 3,4-diisochlorocoumarin, and wherein the amidolytic and/or proteolytic activities of gingipain-1 are stabilized by Ca^{2+} and wherein the amidolytic and/or proteolytic activities of said gingipain-1 are stimulated by glycine-containing peptides and glycine analogues.

An exemplified Arg-gingipain described and termed Arg-gingipain-2 herein exists in the native form in a high molecular

weight form, having an apparent molecular mass of 95 kDa as determined by SDS-PAGE, without boiling of samples. When boiled, the high molecular weight form appears to dissociate into components of 50 kDa, 43 kDa, 27 kDa and 17 kDa. Arg-gingipain-2 is the name given to the 50 kDa, enzymatically active component of the high molecular weight complex.

The complete amino acid sequence of an exemplified mature Arg-gingipain is given in SEQ ID NO:11, from amino acid 228 through amino acid 719. A second possible exemplary amino acid sequence is given in SEQ ID NO:4, amino acids 1 through 510. In nature these proteins are produced by the archebacterium Porphyromonas gingivalis; it can be purified from cells or from culture supernatant or as a recombinant expression product using the methods provided herein. Without wishing to be bound by any theory, it is proposed that these sequences correspond to Arg-gingipain-2.

As used herein with respect to Arg-gingipain-1, a substantially pure Arg-gingipain preparation means that there is only one protein band visible after silver-staining an SDS polyacrylamide gel run with the preparation, and the only amidolytic and/or proteolytic activities are those with specificity for L-arginine in the P₁ position relative to the bond cleaved. A substantially pure high molecular weight Arg-gingipain preparation has only one band (95 kDa) on SDS-PAGE (sample not boiled) or four bands (50 kDa, 43 kDa, 27 kDa, 17 kDa; sample boiled). No amidolytic or proteolytic activity for substrates with lysine in the P₁ position is evident in a substantially pure high molecular weight or Arg-gingipain-2 preparation. Furthermore, a substantially pure preparation of Arg-gingipain has been separated from components with which it occurs in nature. Substantially pure Arg-gingipain is substantially free of naturally associated components when separated from the native contaminants which accompany them in their natural state. Thus, Arg-gingipain that is chemically synthesized or recombinantly synthesized in a cellular system

different from the cell from which it naturally originates will be substantially free from its naturally associated components. Techniques for synthesis of polypeptides are described, for example, in Merrifield (1963) *J. Amer. Chem. Soc.*, 85, 2149-2156.

5

A chemically synthesized Arg-gingipain protein is considered an "isolated" polypeptide, as is an Arg-gingipain produced as an expression product of an isolated proteinase-encoding polynucleotide which is part of an expression vector (i.e., a "recombinant proteinase"), even if expressed in a homologous cell type.

10

Recombinant Arg-gingipain-1, Arg-gingipain-2 and HMW Arg-gingipain can be obtained by culturing host cells transformed with the recombinant polynucleotides comprising nucleotide sequences encoding an Arg-gingipain as described herein under conditions suitable to attain expression of the proteinase-encoding sequence.

15

Example 1 below and Chen et al. (1992) supra describe the purification of Arg-gingipain-1 and HMW Arg-gingipain from P. gingivalis culture supernatant, i.e., from a natural source. Various methods for the isolation of an Arg-gingipain from other biological material, such as from nonexemplified strains of P. gingivalis or from cells transformed with recombinant polynucleotides encoding such proteins, may be accomplished by methods known in the art. Various methods of protein purification are known in the art, including those described, e.g., in Guide to Protein Purification, ed. Deutscher, Vol. 182 of Methods in Enzymology (Academic Press, Inc.: San Diego, 1990) and Scopes, Protein Purification: Principles and Practice (Springer-Verlag: New York, 1982).

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Chromatography over Sephadex G-150 yielded four peaks with Bz-L-Arg-pNA-hydrolyzing activity. In each of these fractions, the hydrolytic activity was dependent on cysteine and enhanced many-fold by the addition of glycyl-glycine or glycine amide.

35

Antibody specific for Arg-gingipain-1 immunoprecipitates proteinase from all four Sephadex G-150 peaks. Without wishing to be bound by any particular theory, it is postulated that the four-peak Bz-L-Arg-pNA-amidolytic profile is an anomaly resulting from the binding of gingipain-1 to membrane or nucleic acid fragments. Alternatively, those peaks containing higher molecular weight protein may contain partially processed gingipain-1 precursors. Although the purification of gingipain-1 as exemplified is from extracellular protein, it can also be purified from the bacterial cells.

Further analysis (see Example 1) of the high molecular weight fractions containing Arg-specific amidolytic and proteolytic activity revealed that Arg-gingipain-2 (50 kDa) occurred non-covalently bound to proteins of 44 kDa, 27 kDa and 17 kDa, which have hemagglutinin activity. The empirically determined N-terminal amino acid sequence of the complexed 44 kDa protein corresponds to amino acids 720-736 of SEQ ID NO:11.

Arg-Gingipain-1 was further purified from the Sephadex G-150 Peak 4 protein mixture by further steps of anion exchange chromatography over DEAE-cellulose and two runs over Mono S FPLC. Arg-gingipain-1 recovery was markedly reduced if an affinity chromatography step (L-Arginyl-Sepharose 4B) was used to remove trace amounts of a contaminating proteinase with specificity for cleavage after lysine residues.

Purified Arg-gingipain-1 exhibits an apparent molecular mass of about 50 kDa as determined by SDS-polyacrylamide gel electrophoresis. The size estimate obtained by gel filtration on Superose 12 (Pharmacia, Piscataway, NJ) is 44 kDa. Amino-terminal sequence analysis through 43 residues gave a unique structure which showed no homology with any other proteins, based on a comparison in the protein NBRF data base, release 39.0. The sequence obtained is as follows:

Tyr-Thr-Pro-Val-Glu-Glu-Lys-Gln-Asn-Gly-Arg-Met-Ile-Val-Ile-Val-Ala-Lys-Lys-Tyr-Glu-Gly-Asp-Ile-Lys-Asp-Phe-Val-Asp-Trp-Lys-Asn-

Gln-Arg-Gly-Leu-Thr-Lys-Xaa-Val-Lys-Xaa-Ala (SEQ ID NO:1).

The C-terminal amino acid sequence of the gingipain-1 (major form recognized in zymography SDS-PAGE, 0.1% gelatin in gel), was found to be Glu-Leu-Leu-Arg. (SEQ ID NO:5). This corresponds to the amino acids 716-719 in SEQ ID NO:4 and nucleotides 3094-3105 in SEQ ID NO:3. This is consistent with the model for autoproteolytic processing of the precursor polyprotein to produce the mature 50 kDa gingipain-1 protein.

Comparison of SEQ ID NO:1 with SEQ ID NO:4 and 11 shows differences at amino acids 37-38 of the mature Arg-gingipain. Without wishing to be bound by any theory, it is proposed that SEQ ID NO:3 (or SEQ ID NO:10) comprises the coding sequence for Arg-gingipain-2, the enzymatically active component of the high molecular weight form of Arg-gingipain. This is consistent with the observation that there are at least two genes with substantial nucleic acid homology to the Arg-gingipain-specific probe.

The enzymatic activity of Arg-gingipain-1 is stimulated by glycine and glycine-containing compounds. In the absence of a glycine-containing compound, the enzyme has essentially the same amidolytic activity in the pH range 7.5-9.0. However, in the presence of glycyl-glycine, e.g., substantial sharpening of the pH range for activity is observed, with the optimum being between pH 7.4 and 8.0. Preliminary kinetic data indicate that the effect of glycine and glycine analogues is to raise both k_{cat} and K_m equally so that the k_{cat}/K_m ratio does not change. It is therefore likely that these compounds bind to the enzyme and/or substrate after an enzyme-substrate complex has already formed. The high molecular weight form is stimulated only about half as much by glycine compounds.

Arg-gingipain-1 requires cysteine for full amidolytic activity, and, although it is stimulated by other thiol-containing compounds, the effect was less pronounced. Cysteine and

cysteamine are most efficient, presumably because they perform the dual roles of reducing agents and glycine analogues.

5 The amidolytic activity of Arg-gingipain-1 is inhibited by a number of -SH blocking group reagents, oxidants, Ca^{2+} chelating agents, and Zn^{2+} . The effect of chelating agents EDTA and EGTA was reversed completely by the addition of excess Ca^{2+} , whereas in the case of Zn^{2+} , it was necessary to add o-phenanthroline prior to Ca^{2+} .

10 Typical serine proteinase group-specific inhibitors have no effect on enzyme activity, and it is likely that inhibition by both TLCK and TPCK was caused by reaction with an essential cysteine residue in the enzyme, a known property of chloromethyl ketone derivatives. Significantly, Arg-gingipain-1 was inhibited
15 by such cysteine proteinase inhibitors as trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane, leupeptin and antipain. Although the reactions were not stoichiometric, the inhibition was concentration-dependent. However, human cystatin C, an
20 inhibitor of mammalian and plant cysteine proteinases, does not inhibit Arg-gingipain-1, nor did any of the trypsin-specific inhibitors from human plasma, including $\alpha 2$ -macroglobulin, $\alpha 1$ -proteinase inhibitor, antithrombin III, and $\alpha 2$ -antiplasmin. Indeed, preliminary investigations actually suggested that the
25 inhibitor in each case was being inactivated by Arg-gingipain-1.

Calcium ion stabilizes Arg-gingipain-1 without directly affecting activity. With Ca^{2+} present the enzyme is stable in the pH range between 4.5 and 7.5 for several days at 4°C.
30 However, below pH 4.0 or in the absence of Ca^{2+} , enzyme activity is quickly lost. At 37°C Ca^{2+} considerably increases stability, although activity is lost more rapidly than at the lower temperature. At -20°C Arg-gingipain-1 is stable for several months. During lyophilization, however, it irreversibly loses
35 more than 90% of its catalytic activity.

The amidolytic activity of the purified Arg-gingipain-1 on synthetic peptide substrates was limited to substrates with a P₁-Arg residue. Even then Arg-gingipain-1 had significantly different turnover rates on individual substrates, being most effective against S-2238 (D-Phe-Pip-Arg-pNA) and S-2222 (Bz-Ile-Glu-(γ -OR)-Gly Arg-pNA). Lesser, comparable activity was observed using S2288 (D-Ile-Pro-Arg-pNA) and Bz-Arg-pNA. D-Val-Leu-Lys-pNA (S-2251), Suc-Ala-Ala-Ala-pNA, MeO-Suc-Ala-Ala--Pro-Val-pNA, Suc-Ala-Ala-Pro-Phe-pNA, Gly-Pro-pNA and Cbz-Phe-Leu-Glu-pNA had essentially no substrate activity. This narrow specificity was confirmed by examination of the cleavage products after incubation with the insulin B chain or mellitin; it was found that cleavage occurred specifically after only Arg residues, but not after Lys or any other amino acids unless the last affinity chromatography step over L-Arginine-Sepharose 4B was omitted.

Because progressive periodontitis is characterized by tissue degradation, collagen destruction and a strong inflammatory response, and because P. gingivalis was known to exhibit complement-hydrolyzing activity, purified Arg-gingipain-1 was tested for proteinase activity using purified human complement C3 and C5 as substrates (See Wingrove et al. (1992) J. Biol. Chem. 267: 18902-18907). Low molecular weight Arg-gingipain selectively cleaved the α -chain, generating what initially appeared to be the α' -chain of C3b. Further breakdown fragments of the C3 α' -chain were observed and a decreasing intensity of the α' -band suggested that degradation continued. Visual evidence suggested that the C3 β -chain is resistant to this proteinase. Attempts to demonstrate C3a biological activity in the C3 digestion mixture were unsuccessful, and the C3a-like fragment released from the α -chain was extensively degraded by Arg-gingipain-1.

Human C5 was also digested by Arg-gingipain-1, with initial cleavage specific for the C5 α -chain, as in the case of C3. The α -1 (86 kDa) and the α -2 (30 kDa) fragments were the first

polypeptides to be formed from cleavage of C5 by gingipain-1, and they equal the molecular weight of the intact α -chain, a fragment in the size range of C5a was observed. C5a is more resistant to the Arg-gingipain-1 than C3a, and functional C5a may accumulate without further appreciable degradation. C5a biological activity was detected after digestion of human C5 with Arg-gingipain-1. Characteristic morphologic changes in human neutrophils, known as polarization, were scored by counting deformed cells relative to normally rounded cells.

To test for in vivo biological activity, the purified low molecular weight Arg-gingipain enzyme was injected into guinea pig skin. It induced vascular permeability enhancement at concentrations greater than 10^{-8} M in dose-dependent and proteolytic activity dependent manners. Vascular permeability enhancement activity was not inhibited by diphenhydramine (an antihistamine), and the activity was enhanced by SQ 20,881 (angiotensin-converting enzyme inhibitor). The vascular permeability enhancement by Arg-gingipain-1 was inhibited by soybean trypsin inhibitor (SBTI) at a concentration of 10^{-5} M, a concentration at which SBTI did not inhibit enzymatic activity, as measured with Bz-L-Arg-pNA and azocasein as the substrates.

Human plasma or guinea pig plasma treated with Arg-gingipain-1 (10^{-8} to 10^{-6} M) induced vascular permeability enhancement in the guinea pig skin assay. Vascular permeability enhancement by Arg-gingipain-1 treated plasma was increased by addition of 1,10-phenanthroline (kinase inhibitor, chelating agent for Zn ions) to a final concentration of 1 mM. Vascular permeability enhancement by Arg-gingipain-1 treated plasmas was markedly reduced when plasmas deficient in Hageman factor, prekallikrein or high molecular weight kininogen were used. These results indicate that vascular permeabilizing enhancement by Arg-gingipain-1 acts via activation of Hageman factor and the subsequent release of bradykinin from high molecular weight kininogen by kallikrein.

Intradermal injection of Arg-gingipain-1 in the guinea pig also resulted in neutrophil accumulation at the site of injection, an activity which was dependent on proteolytic activity.

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The foregoing results demonstrate the ability of Arg-gingipain to elicit inflammatory responses in a guinea pig animal model.

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Recombinant Arg-gingipain is useful in methods of identifying agents that modulate Arg-gingipain proteinase activity, whether by acting on the proteinase itself or preventing the interaction of a proteinase with a protein in gingival area, such as C3 or C5. One such method comprises the steps of incubating a proteinase with a putative therapeutic, i.e., Arg-gingipain-inhibiting, agent; determining the activity of the proteinase incubated with the agent; and comparing the activity obtained in step with the activity of a control sample of proteinase that has not been incubated with the agent.

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SDS-PAGE analysis (without boiling) of the purified high molecular weight form of Arg-gingipain revealed a single band of apparent molecular mass of 95 kDa. This estimate was confirmed by analytical chromatography over a TSK 3000SW gel filtration column. When the enzyme preparation was boiled before SDS-PAGE, however, bands of apparent molecular masses of approximately 50 kDa, 44 kDa, 27 kDa and 17 kDa were observed. These bands were not generated by treatments at temperatures below boiling, by reducing agents or detergents. It was concluded that the 95 kDa band was the result of strong non-covalent binding between the lower molecular weight proteins.

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The 50 kDa proteolytic component of the high molecular weight Arg-gingipain was characterized with respect to N-terminal amino acid sequence over 22 amino acids. The sequence was identical to the first 22 amino acids of the 50 kDa, low molecular weight Arg-gingipain-1. Characterization of the high

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molecular weight Arg-gingipain activity showed the same dependence on cysteine (or other thiols) and the same spectrum of response to potential inhibitors. Although the high molecular weight Arg-gingipain was stimulated by glycine compounds, the response was only about half that observed for the low molecular weight form.

The primary structure of the NH₂-terminus of low molecular weight Arg-gingipain determined by direct amino acid sequencing. (SEQ ID NO:1) was used to prepare a mixture of synthetic primer oligonucleotides GIN-1-32 (SEQ ID NO:6) coding for amino acids 2 to 8 of the mature protein and primer GIN-2-30 (SEQ ID NO:7) coding for amino acids 25-32 of the mature protein. These primers were used in PCR on P. gingivalis DNA. A single 105-base pair product (P105) resulted. This was cloned into pCR-ScriptTMSK(-) (Stratagene) and sequenced. Sequence analysis of P105 generated 49 nucleotides from an Arg-gingipain coding sequence. On the basis of the sequence of P105, another primer (GIN-8S-48) SEQ ID NO:8 corresponding to the coding strand of the partial Arg-gingipain gene (48-mers) was synthesized in order to screen the λ DASH DNA library using a ³²P-labeled GIN-8S-48 probe. A partial sequence of the Arg-gingipain gene (nucleotides 1-3159, SEQ ID NO:3) was determined by screening the P. gingivalis DNA library using ³²P-labeled hybridization GIN-8S-48 probe (SEQ ID NO:8). From a total of 2x10⁵ independent plaques screened, seven positive clones were isolated and purified. After extraction and purification, the DNA was analyzed by restriction enzymes: One clone (A1) has a 3.5 kb *Bam*HI fragment and a 3 kb *Pst*I fragment; another clone (B1) has a 9.4 kb *Bam*HI fragment and a 9.4 kb *Pst*I fragment; and 5 clones have a 9.4 kb *Bam*HI fragment and a 10 kb *Pst*I fragment. These results are similar to those obtained by Southern analysis of P. gingivalis DNA and are consistent with the existence of at least two Arg-gingipain genes. The A1 clone was chosen for sequencing because the expected DNA size to encode a 50-kDa protein is approximately 1.35 kb. The 3.159 kb *Pst*I/*Bam*HI fragment from clone A1 was subsequently subcloned into pBluescript SK(-) as a *Pst*I fragment and a *Sma*I/*Bam*HI fragment

and into M13mp18 and 19 as a *Pst*I fragment and a *Pst*I/*Bam*HI fragment and sequenced. In order to clone the stop codon of gingipain-1, which was missing in the *Pst*I/*Bam*HI fragment, *Pst*I/*Hind*III double digested *P. gingivalis* DNA clones were hybridized with ³²P-labeled GIN-14-20 (SEQ ID NO:9) (nucleotides 2911-2930 of SEQ ID NO:3) localized at the 3' end of this clone. A *Pst*I/*Hind*III fragment of approximately 4.3 kb was identified and cloned into pbluescript SK(-). Smaller fragment (*Pst*I/*Asp*713 and *Bam*HI/*Hind*III) was also subcloned into M13mp18 and 19.

SEQ ID NO:3 is the DNA sequence of the 3159 bp *Pst*I/*Bam*HI fragment (see Table 1).

TABLE 1
Nucleotide sequence and deduced
amino acid sequence of an Arg-gingipain

10	20	30	40
CTG CAG AGG GGT GGT AAA GAC CGC CTC GGG ATC GAG GCG TTT GAC ACC			
GAC GTC TCC CGA CCA TTT CTG GCG GAG CCC TAG CTC CGG AAA CTC TGC			
50	60	70	80
GCG ACA AGC CGC CGC AGC CTC CTC TTC GAA GGT GTC TCG AAC GTC CAC			
CCG TGT TCG GCG GCG TCG GAG GAG AAG CTT CCA CAG AGC TTG CAG GTG			
100	110	120	130
ATC GGT GAA TCC GTA GCA GTG CTC ATT GCC ATT GAG CAG CAC CGA GGT			
TAG CCA CTT AGG CAT CGT CAC GAG TAA CGG TAA CTC GTC GTG GCT CCA			
150	160	170	180
GTG GCG CAT CAG ATA TAT TTT CAT CAG TGG ATT ATT AGG GTA TCG GTC			
CAC CGC GTA GTC TAT ATA AAA GTA GTC ACC TAA TAA TCC CAT AGC CAG			
200	210	220	230
AGA AAA AGC CTT CCG AAT CCG ACA AAG ATA GTA GAA AGA GAG TGC ATC			
TCT TTT TCG GAA GCG TTA GCG TGT TTC TAT CAT CTT TCT CTC ACG TAG			
250	260	270	280
TGA AAA CAG ATC ATT CGA GGA TTA TCG ATC AAC TGA AAA GCG AGG AGT			
ACT TTT GTC TAG TAA GCT CCT AAT AGC TAG TTG ACT TTT CCG TCC TCA			
290	300	310	320
TGT TTT GCG TTT TGG TTC GGA AAA TTA CCT GAT CAG CAT TCG TAA AAA			
ACA AAA GCG AAA ACC AAG CCT TTT AAT GGA CTA CTC GTA AGC ATT TTT			
340	350	360	370
CCT GCG GCG AGA ATT TTT TCG TTT TGG CCG GAG AAT TAA AAA TTT TTC			
GCA CCG CCG TCT TAA AAA AGC AAA ACC GCG CTC TTA ATT TTT AAA AAC			
390	400	410	420
GAA CCA CAG CGA AAA AAA TCT CGC GCC GTT TTC TCA GGA TTT ACA GAC			
CTT GGT GTC GCT TTT TTT AGA GCG CCG CAA AAG AGT CCT AAA TGT CTG			
440	450	460	470
CAC AAT CCG AGC ATT TTC GGT TCG TAA TTC ATC GAA GAG ACA GGT TTT			
GTG TTA CCG TCG TAA AAG CCA AGC ATT AAG TAG CTT CTC TGT CCA AAA			
490	500	510	520
ACC GCA TCG AAA TCA GAG AGA GAA TAT CCG TAG TCC AAC GGT TCA TCC			
TGG CGT AAC TTT AGT CTC TCT CTT ATA GCG ATC AGG TTG CCA AGT AGG			
530	540	550	560
TTA TAT CAG AGG TTA AAA GAT ATG GTA CGC TCA TCG AGG AGC TGA TTG			
AAT AEA GTC TCC AAT TTT CTA TAC CAT GCG AGT AGC TCC TCG ACT AAC			
580	590	600	610
GCT TAG TAG GTC AGA CTT TCT TAA GAG ACT ATC GCG ACC TAC AGG AAG			
CCA ATC ATC CAC TCT GAA AGA ATT CTC TCA TAG CCG TGG ATG TCC TTC			
630	640	650	660
TTC ATG GCA CAC AAG CCA AAG GAG GCA ATC TTC GCA GAC CCG ACT CAT			
AAG TAC CGT GTG TTC CGT TTC CTC CGT TAG AAG CBT CTC GCG TGA GTA			

TABLE 1 (cont'd)

21

680	690	700	710	720
ATC AAA AGG ATC AAA CGA CTT TTC CAT ACG ACA ACC AAA TAG CCG TCT				
TAG TTT TCC TAC TTT GCT GAA AAG GTA TGC TGT TGG TTT ATC GGC AGA				
730	740	750	760	
ACG GTA GAC GAA TGC AAA CCC AAT ATG AGG CCA TCA ATC AAT CCG AAT				
TGC CAT CTG CTT ACG TTT GCG TTA TAC TCC GGT AGT TAC TTA GGC TTA				
770	780	790	800	810
GAC AGC TTT TGG GCA ATA TAT TAT GCA TAT TTT GAT TCG CGT TTA AAG				
CTG TCG AAA ACC CGT TAT ATA ATA CGT ATA AAA CTA AGC GCA AAT TTC				
820	830	840	850	860
GAA AAG TGC ATA TAT TTG CGA TTG TGC TAT TTC TTT CCG TTT CTA TGT				
CTT TTC ACG TAT ATA AAC GCT AAC ACC ATA AAG AAA GGC AAA GAT ACA				
870	880	890	900	910
GAA TTT TGT CTC CCA AGA AGA CTT TAT AAT GCA TAA ATA CAG AAG GGG				
CTT AAA ACA GAG GGT TCT TCT GAA ATA TTA COT ATT TAT GTC TTC CCC				
920	930	940	950	960
TAC TAC ACA GTA AAA TCA TAT TCT AAT TTC ATC AAA ATG AAA AAC TTG				
ATG ATG TGT CAT TTT AGT ATA AGA TTA AAG TAG TTT TAC TTT TTG AAC				
			M	K N L
970	980	990	1000	
AAC AAG TTT GTT TCG ATT GGT CTT TGC TCT TCC TTA TTA GGA GGA ATG				
TTG TTC AAA CAA AGC TAA CCA GAA ACG AGA AGG AAT AAT CCT CCT TAC				
N K F V S I A L C S S L L G G H				
1010	1020	1030	1040	1050
GCA TTT GCG CAG CAG ACA GAG TTG GGA CGC AAT CCG AAT GTC AGA TTG				
CGT AAA CGC GTC GTC TGT CTC AAC CCT GCG TTA GGC TTA CAG TCT AAC				
A F A Q Q T E L G R N P N V R L				
1060	1070	1080	1090	1100
CTC GAA TCC ACT CAG CAA TCG GTC ACA AAG GTT CAG TTC CGT ATG GAC				
GAG CTT AGG TGA CTC CTT ACC CAC TGT TTC CAA GTC AAG GCA TAC CTG				
L E S T Q Q S V T K V Q F R M D				
1110	1120	1130	1140	1150
AAC CTC AAG TTC ACC GAA GTT CAA ACC CCT AAG GGA ATC GGA CAA GTG				
TTG GAG TTC AAG TCG CTT CAA GTT TCG GGA TTC CCT TAG CCT GTT CAC				
N L R F T E V Q T P R G I G Q V				
1160	1170	1180	1190	1200
CCG ACC TAT ACA GAA GGG GTT AAT CTT TCC GAA AAA GGG ATG CCT ACC				
GCG TCG ATA TGT CTT CCC CAA TTA GAA AGG CTT TTT CCC TAC GGA TCG				
P T Y T E G V N L S E R G H P T				
1210	1220	1230	1240	
CTT CCC ATT CTA TCA CGC TCT TTG GCG GTT TCA GAC ACT CGT GAG ATG				
GAA GCG TAA GAT AGT GCG AGA AAC CGC CAA AGT CTG TGA GCA CTC TAC				
L P I L S R S L A V S D T R E H				
1250	1260	1270	1280	1290
AAG CTA GAG GTT GTT TCC TCA AAG TTC ATC GAA AAG AAA AAT GTC CTG				
TTG CAT CTC CAA CAA AGG AGT TTC AAG TAG CTT TTC TTT TTA CAG GAC				
K V E V V S S K F I E K K N V L				

TABLE 1 (cont'd)

22

1300	1310	1320	1330	1340
ATT GCA CCC TCC AAG GGC ATG ATT ATG CGT AAC GAA GAT CCG AAA AAG				
TAA CGT GGC AGG TTC CCG TAC TAA TAC GCA TTG CTT CTA GGC TTT TTC				
I A P S K G M I M R N E D P K K				
1350	1360	1370	1380	1390
ATC CCT TAC GTT TAT GGA AAG AGC TAC TCG CAA AAC AAA TTC TTC CCG				
TAG GGA ATG CAA ATA CCT TTC TCG ATG AGC GTT TTG TTT AAG AAG GGC				
I P Y V Y G K S Y S Q N K F F P				
1400	1410	1420	1430	1440
GGA GAG ATC GCC ACG CTT GAT GAT CCT TTT ATC CTT CGT GAT GTG CGT				
CCT CTC TAG CCG TGC GAA CTA CTA GGA AAA TAG GAA GCA CTA CAC GCA				
G E I A T L D D P F I L R D V R				
1450	1460	1470	1480	
GGA CAG GTT GTA AAC TTT GCG CCT TTG CAG TAT AAC CCT GTG ACA AAG				
CCT GTC CAA CAT TTG AAA CCG GGA AAC GTC ATA TTG GGA CAC TGT TTC				
G Q V V N F A P L Q Y N P V T K				
1490	1500	1510	1520	1530
ACG TTG CCG ATC TAT ACC GAA ATC ACT GTG GCA GTG AGC GAA ACT TCG				
TGC AAC GCG TAG ATA TGC CTT TAG TGA CAC CGT CAC TCG CTT TGA AGC				
T L R I Y T E I T V A V S E T S				
1540	1550	1560	1570	1580
GAA CAA GGC AAA AAT ATT CTC AAC AAG AAA CGT ACA TTT GCC GGC TTT				
CTT GTT CCG TTT TTA TAA GAC TTG TTC TTT CCA TGT AAA CCG CCG AAA				
E Q G K N I L N K K G T F A G F				
1590	1600	1610	1620	1630
GAA GAC ACA TAC AAG CCG ATG TTC ATG AAC TAC GAG CCG GCG CGT TAC				
CTT CTC TGT ATG TTC GCG TAC AAG TAC TTG ATG CTC GCG CCC GCA ATG				
E D T Y K R M F M N Y E P G R L				
1640	1650	1660	1670	1680
ACA CCG GTA GAG GAA AAA CAA AAT GGT CGT ATG ATC GTC ATC GTA GCC				
TGT GGC CAT CTC CTT TTT GTT TTA CCA GCA TAC TAG CAG TAG CAT CCG				
T P V E E K Q N G R M I V A				
1690	1700	1710	1720	
AAA AAG TAT GAC GGA GAT ATT AAA GAT TTC GTT GAT TGG AAA AAC CAA				
TTT TTC ATA CTC CCT CTA TAA TTT CTA AAG CAA CTA ACC TTT TTG GTT				
K K Y F G D I K D F V D W K N Q				
1730	1740	1750	1760	1770
CGC GGT CTC CGT ACC GAG GTG AAA CTC GCA GAA GAT ATT GGT TGT CCG				
CGC CCA GAG GCA TGG CTC CAC TTT CAC CGT CTT CTA TTA CCA AGA GCG				
R G L R T E V K V A E D I A S P				
1780	1790	1800	1810	1820
GTT ACA GGT AAT GGT ATT CAG CAG TTC GTT AAG CAA GAA TAC GAG AAA				
CAA TGT CCA TTA CGA TAA GTC AAC CAA TTC CTT CTT ATG CTC TTT				
V T A N A I Q F V K Q E Y E K				
1830	1840	1850	1860	1870
GAA GGT AAT GAT TTG ACC TAT GTT CTT TTG GTT GCC GAT CAC AAA GAT				
CTT CCA TTA CTA AAC TGG ATA CAA GAA AAC CAA CCG CTA GTG TTT CTA				
E G N D L F Y V L L V G D H K D				

TABLE 1 (cont'd)

23

1880	1890	1900	1910	1920
ATT GGT GGC AAA	ATT ACT CCG GGG	ATC AAA TCC GAC	CAG GTA TAT GGA	
TAA GGA CGC TTT	TAA TGA GGC CCC	TAG TTT AGG CTG	GTC CAT ATA CTT	
I P A R I T P G I K S D Q V Y G				
1930	1940	1950	1960	
CAA ATA GTA GGT	AAT GAC CAC TAC	AAC GAA GTC TTC	ATC GGT GGT TTC	
TTT TAT CAT CCA	TTA CTC GTC AGC	TTG CTT CAG AAG	TAG CCA GCA AAG	
Q I V G N D H Y N E V F I G R F				
1970	1980	1990	2000	2010
TCA TGT GAC AGC	AAA CAG GAT CTG	AAG ACA CAA ATC	GAT CGG ACT ATT	
AGT ACA CTC TCG	TTT CTC CTA GAC	TTC TGT GTT TAG	CTA GCC TGA TAA	
S C E S K E D L K T Q I D R T I				
2020	2030	2040	2050	2060
CAC TAT GAG CGC	AAT ATA ACC	ACG GAA GAC	AAA TGG CTC	GGT CAG GCT
GTG ATA CTC GCG	TTA TAT TGG	TGC CTT CTG	TTT ACC GAG	CCA GTC CGA
H Y E R N I T T E D K W L G Q A				
2070	2080	2090	2100	2110
CTT TGT ATT GCT	TCG GGT GAA GGA	GGC CCA TCC	GCA GAC AAT	GGT GAA
GAA ACA TAA CGA	AGC CGA CTT CTT	CCG GGT AGG	CGT CTG TTA	CCA CTT
L C I A S A E G P S A D N G E				
2120	2130	2140	2150	2160
AGT GAT ATC CAG	CAT GAG AAT	GTA ATC GCC	AAT CTG CTT	ACC CAG TAT
TCA CTA TAG GTC	GTA CTC TTA	CAT TAG CCG	TTA GAC GAA	TGG GTC ATA
S D I Q H E N V I A N L L T Q Y				
2170	2180	2190	2200	
GGC TAT ACC AAG	ATT ATC AAA	TGT TAT GAT	CCG GGA GTA	ACT CCT AAA
CCG ATA TGG TTC	TAA TAG TTT	ACA ATA CTA	GGC COT	CAT TGA GGA TTT
G Y T K I I K C Y D P G V T P K				
2210	2220	2230	2240	2250
AAC ATT ATT GAT	GCT TTC AAC	GGA GGA ATC	TCG TTG GTC	AAC TAT ACG
TTG TAA TAA CTA	CGA AAG TTG	CCT CCT TRG	AGC AAC CAG	TTG ATA TGC
N I I D A F N G G I S L V N Y T				
2260	2270	2280	2290	2300
GGC CAC GGT AGC	GAA ACA GCT	TGG GGT ACG	TCT CAC TTC	GGC ACC ACT
CCG GTG CCA TCG	CTT TGT CCA	ACC CCA TGC	AGA GTG AAG	CCG TGG TGA
G H C S E T A W G T S H F G T T				
2310	2320	2330	2340	2350
CAT GTC AAG CAG	CTT ACC AAC	AGC AAC CAG	CTA CCG TTT	ATT TTC GAC
GTA CAC TTC GTC	GAA TGG TTG	TCG TTG GTC	GAT GGC AAA	TAA AAG CTG
H V K Q L T N S N Q L P F I F D				
2360	2370	2380	2390	2400
GTA GCT TGT GTG	AAT GGC GAT TTC	CTA TTC AGC	ATC COT TGC	TTG GCA
CAT CGA ACA CAC	TTA CCG CTA	AAG GAT AAG	TCG TAC GGA	ACG AAG COT
V A C V N G D F L F S M P C F A				
2410	2420	2430	2440	
GAA GGC CTG ATC	CCT GCA CAA	AAA GAT GGT	AAG CCG	ACA GGT
CTT CCG GAC TAC	GCA CST GTT	TTT CTA CCA	TTG GGC	TGT CCA
E A L H R A Q K D G K F T G T V				

TABLE 1 (cont'd)

24

2450	2460	2470	2480	2490
GCT ATC AEA GCG TCT ACS ATC AAC CAG TCT TGG GCT TCT CCT ATG CGC	CGA TAG TAT CGC AGA TGC TAG TTG GTC AGA ACC CGA AGA GGA TAC GCG	A I I A S T I N Q S W A S P M R		
2500	2510	2520	2530	2540
GGC CAG GAT GAG ATG AAC GAA ATT CTG TGC GAA AAA CAC CGC AAC AAC	CCC GTC CTA CTC TAC TTG CTT TAA GAC ACG CTT TTT GTG GGC TTG TTG	G Q D E M N E I L C E K H P N N		
2550	2560	2570	2580	2590
ATC AAG CTT ACT TTC GGT GGT GTC ACC ATG AAC GGT ATG TTT GCT ATG	TAG TTC GCA TGA AAG CCA CCA CAG TGG TAC TTG CCA TAC AAA CGA TAC	I K E T F G G V T M N G M F A M		
2600	2610	2620	2630	2640
GTG GAA AAG TAT AAA AAG GAT GGT GAG AAG ATG CTC GAC ACA TGG ACT	CAC CTT TTC AEA TTT TTC CTA CCA CTC TTC TAC GAG CTG TGT ACC TGA	V E K Y K K D G E K M L D T W T		
2650	2660	2670	2680	
GTT TTC GGC GAC CCC TCG CTC GTT CGT ACA CTT GTC CCG ACC AAA	CRA AAG CCG CTC GGC AGC GAC GAG CRA GCA TGT GAA CAG GCG TCG TTT	V F G D P S L L V R T L V P T K		
2690	2700	2710	2720	2730
ATG CAG GTT ACG GCT CCG GCT CAG ATT AAT TTG ACG GAT GCT TCA GTC	TAC GTC CAA TGC CGA GCG CGA GTC TAA TTA AAC TGC CTA CGA AGT CAG	M Q V T A P A Q I N L T D A S V		
2740	2750	2760	2770	2780
AAC GTA TCT TGC GAT TAT AAT GGT GCT ATT GCT ACC ATT TCA GCC AAT	TTG CAT AGA ACG CTA ATA TTA CCA CGA TAA CGA TGG TAA ACT CGC TTA	N V S C D Y N G A T A T I S A N		
2790	2800	2810	2820	2830
GGA AAG ATG TTC GGT TCT GCA GTT GTC GAA AAT GGA ACA GGT ACA ATC	CCT TTC TAC AAG CCA AGA CGT CAA CAG CTT TTA CCT TGT CGA TGT TAG	G K M F G S A V V E N G T A T I		
2840	2850	2860	2870	2880
AAT CTC ACA GGT CTG ACA AAT GAA AGC ACG CTT ACC CTT ACA GTA GTT	TTA GAC TGT CCA GAC TGT TTA CTT TCG TGC GAA TGG GAA TGT CAT CAA	N L T G L T N E S T L T L T V V		
2890	2900	2910	2920	
GGT TAC AAC AAA GAG ACG GTT ATT AAG ACC ATC AAC ACT AAT GGT GAG	CCA ATG TTG TTT CTC TGC CAA TAA TTC TGG TAG TTG TGA TTA CCA CTC	G Y N K E T V I K T I N T N G E		
2930	2940	2950	2960	2970
CCT AAC CCC TAC CAG CCC GTT TCC AAC TTG ACA GCT ACA ACG CAG GGT	GGG TTG GGG ATC CTC GCG CAA AGG TTG AAC TGT CGA TGT TGC GTC CCA	P N P Y Q P V S N L T A T T Q G		
2980	2990	3000	3010	3020
CAG AAA GTA ACG CTC AAG TGG GAT GCA CCC ACG ACG AAA ACC AAT GCA	CTC TTT CAT TGC GAG TTC ACC CTA CGT GCG TCG TCG TTT TCG TTA CGT	Q K V T L K W D A F S T K T N A		

TABLE 1 (cont'd)

25

3030	3040	3050	3060	3070
ACC ACT AAT ACC GCT CGC AGC GTC GAT GGC ATA CCA GAA TTG GTT GTT				
TGG TGA TTA TGG CGA GCG TCG CAC CTA CCG TAT GCT CTT AAC CAA GAA				
T T N T A R S V D G I R E L V L				
3080	3090	3100	3110	3120
CTG TCA GTC AGC GAT GCG CCC GAA CTT CTT CCG AGC GGT CAG GCG CAG				
GAC AGT CAG TCG CTA CCG GCG CTT GAA GAA GCG TCG CCA GTC CCG CTC				
L S V S D A P E L L R E G Q A F				
3130	3140	3150		
ATT GTT CTT GAA GCT CAC GAT GTT TGG AAT GAT GGA TCC				
TAA CAA GAA CTT CCA GTG CTA CCA ACC TTA CTA CCT AGG				
F V L E A H D V W N D G S				

Exemplified nucleotide sequences encoding a mature Arg-gingipain, termed an Arg-gingipain-2 herein, extends from 1630-3105 in SEQ ID NO:3 and in SEQ ID NO:10. The first ATG appears at nucleotide 949 and is followed by a long open reading frame (ORF), of 5111 bp in Table 2 (SEQ ID NO:10). This ORF was the largest one observed. However, the first ATG is following by 8 others in frame (at nucleotides 1006, 1099, 1192, 1246, 1315, 1321, 1603, and 1609). The most likely candidate to initiate translation is currently unknown. Which of these initiation codons are used in translation of the Arg-gingipain-2 precursor can be determined by expression of the polyprotein in bacteria and subsequent amino-terminal sequence analysis of proprotein intermediates. The sequence derived from 5' noncoding sequences is composed of 948 bp. The primary structure of the mature Arg-gingipain molecule can be inferred from the empirical amino-terminal and carboxy-terminal sequences and molecular mass. Thus, mature Arg-gingipain-2 has an amino terminus starting at nucleotide residue 1630 in SEQ ID NO:3 and at amino acid 1 in SEQ ID NO:4. As expected for an arginine-specific protease, the mature protein is cleaved after an arginine residue. The 50 kDa and the 44 kDa bands from Bz-L-Arg-pNa activity peaks have an identical sequence to that deduced amino acid sequence of gingipain, encoded respectively at nucleotides 1630-1695 and at nucleotides 3106-3156. From these data, the carboxyl terminus is most likely derived from autoproteolytic processing after the arginine residue encoded at 3103-3105 where the amino terminus encoding sequence of a hemagglutinin component starts (nucleotide 3106). The deduced 492 amino acids of gingipain-2 give rise to a protease molecule with a calculated molecular weight of 54 kDa which correlates well with the molecular mass of 50 kDa determined by SDS-PAGE analysis. Tables 1 and 2 (see also SEQ ID NO:10 and 11) presents the coding sequence and deduced amino acid sequence of gingipain-2. The first nucleotide presented in the sequence belongs to the *Pst*I cloning site and is referred as nucleotide 1. Bold face letters indicate the potential sites of initiation ATG and the first codon of the mature gingipain-2. The amino terminal sequence of gingipain-2 and the amino terminal

sequence of 44 kDa bands from Bz-L-Arg-pNa activity peaks are underlined.

5 Table 2 (corresponding to SEQ ID NOS:10-11) presents the
nucleotide sequence encoding the complete prepolyprotein
sequence, including both the protease component and the
hemagglutinin component(s) of HMW Arg-gingipain. The coding
sequence extends from an ATG at nucleotide 949 through a TAG stop
codon at nucleotide 6063 in SEQ ID NO:10. The deduced amino acid
10 sequence is given in SEQ ID NO:11.

TABLE 2

28

Sequence Range: 1 to 7266

```
>PstI                               >StuI
|                                   |
CTGCAGAGGG CTGGTAAAGA CCGCCTCGGG ATCGAGGCCT TTGAGACGGG CACAAGCCGC CGCAGCCTCC
                                100
TCTTCGAAGG TGTCTCGAAC GTCCACATCG GTGAATCCGT AGCAGTGCTC ATTGCCATTG AGCAGCACCG
                                200
AGGTGTGGCG CATCAGATAT ATTTTCATCA GTGGATTATT AGGGTATCGG TCAGAAAAAG CCTTCCGAAT
                                >ClaI
                                |
CCGACAAAGA TAGTAGAAG AGAGTGCATC TGAAAACAGA TCATTGAGG ATTATCGATC AACTGAAAAG
                                300
GCAGGAGTTG TTTTGCCTTT TGGTTCGGAA AATTACCTGA TCAGCATTG TAAAAACGTG GCGCGAGAAT
                                400
TTTTTCGTTT TGGCGCGAGA ATTAAAAATT TTTGGAACCA CAGCGAAAAA AATCTCGCGC CGTTTTCTCA
GGATTACAG ACCACAATCC GAGCATTTC GGTTCGTAAT TCATCGAAGA GACAGGTTTT ACCGCATTGA
                                500
AATCAGAGAG AGAATATCCG TAGTCCAACG GTTCATCCTT ATATCAGAGG TTAAAAGATA TGSTACGCTC
                                600
ATCGAGGAGC TGATTGGCTT AGTAGGTGAG ACTTTCTTAA GAGACTATCG GCACCTACAG GAAGTTCATG
                                700
GCACACAAGG CAAAGGAGGC AATCTTCGCA GACCGGACTC ATATCAAAG GATGAAACGA CTTTTCCATA
CGACAACCA ATAGCCGTCT ACGGTAGACG AATGCAAACC CAATATGAGG CCATCAATCA ATCCGAATGA
                                800
CAGCTTTTGG GCAATATATT ATGCATATTT TGATTGCGGT TTAAAGGAAA AGTGCATATA TTTGCGATTG
                                900
TGGTATTCTT TTCGGTTTCT ATGTGAATTT TGTCTCCCAA GAAGACTTTA TAATGCATAA ATACAGAAGS
GGTACTACAC AGTAAATCA TATTCTAATT TCATCAAA ATG AAA AAC TTG AAC AAG TTT GTT TCG
                                M K N L N K F V S>
                                1000
ATT GCT CTT TGC TCT TCC TTA TTA GGA GGA ATG GCA TTT GCG CAG CAG ACA GAG TTG
I A L C S S L L G G M A F A Q Q T E L>
GGA CGC AAT CCG AAT GTC AGA TTG CTC GAA TCC ACT CAG CAA TCG GTG ACA AAG GTT
G R N P N V R L L E S T Q Q S V T K V>
                                1100
CAG TTC CGT ATG GAC AAC CTC AAG TTC ACC GAA GTT CAA ACC CCT AAG GGA ATC GGA
Q F R M D N L K F T E V Q T P K G I G>
                                1200
```

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Table 2 (contd.)

29

CAA GTG CCG ACC TAT ACA GAA GGG GTT AAT CTT TCC GAA AAA GGG ATG CCT ACG CTT
Q V P T Y T E G V N L S E K G M P T L>

CCC ATT CTA TCA CGC TCT TTG GCG GTT TCA GAC ACT CGT GAG ATG AAG GTA GAG GTT
P I L S R S L A V S D T R E M K V E V>

1300

GTT TCC TCA AAG TTC ATC GAA AAG AAA AAT GTC CTG ATT GCA CCC TCC AAG GGC ATG
V S S K F I E K K N V L I A P S K G M>

ATT ATG CGT AAC GAA GAT CCG AAA AAG ATC CCT TAC GTT TAT GGA AAG AGC TAC TCG
I M R N E D P K K I P Y V Y G K S Y S>

1400

CAA AAC AAA TTC TTC CCG GGA GAG ATC GCC ACG CTT GAT GAT CCT TTT ATC CTT CGT
Q N K F F P G E I A T L D D P F I L R>

GAT GTG CGT GGA CAG GTT GTA AAC TTT GCG CCT TTG CAG TAT AAC CCT GTG ACA AAG
D V R G Q V V N F A P L Q Y N P V T K>

1500

ACG TTG CGC ATC TAT ACG GAA ATC ACT GTG GCA GTG AGC GAA ACT TCG GAA CAA GGC
T L R I Y T E I T V A V S E T S E Q G>

1600

AAA AAT ATT CTG AAC AAG AAA GGT ACA TTT GCC GGC TTT GAA GAC ACA TAC AAG CGC
K N I L N K K G T F A G F E D T Y K R>

ATG TTC ATG AAC TAC GAG CCG GGG CGT TAC ACA CCG GTA GAG GAA AAA CAA AAT GGT
M F M N Y E P G R Y T P V E E K Q N G>

1700

CGT ATG ATC GTC ATC GTA GCC AAA AAG TAT GAG GGA GAT ATT AAA GAT TTC GTT GAT
R M I V I V A K K Y E G D I K D F V D>

TGG AAA AAC CAA CGC GGT CTC CGT ACC GAG GTG AAA GTG GCA GAA GAT ATT GCT TCT
W K N Q R G L R T E V K V A E D I A S>

1800

CCC GTT ACA GCT AAT GCT ATT CAG CAG TTC GTT AAG CAA GAA TAC GAG AAA GAA GGT
P V T A N A I Q Q F V K Q E Y E K E G>

AAT GAT TTG ACC TAT GTT CTT TTG GTT GGC GAT CAC AAA GAT ATT CCT GCC AAA ATT
N D L T Y V L L V G D H K D I P A K I>

1900

ACT CCG GGG ATC AAA TCC GAC CAG GTA TAT GGA CAA ATA GTA GGT AAT GAC CAC TAC
T P G I K S D Q V Y G Q I V G N D H Y>

2000

AAC GAA GTC TTC ATC GGT CGT TTC TCA TGT GAG AGC AAA GAG GAT CTG AAG ACA CAA
N E V F I G R F S C E S K E D L K T Q>

>Clal

ATC GAT CGG ACT ATT CAC TAT GAG CGC AAT ATA ACC ACG GAA GAC AAA TGG CTC GGT
I D R T I H Y E R N I T T E D K W L G>

RECTIFIED SHEET (RULE 91)

Table 2 (contd.)

30

2100
CAG GCT CTT TGT ATT GCT TCG GCT GAA GGA GGC CCA TCC GCA GAC AAT GGT GAA AGT
Q A L C I A S A E G G P S A D N G E S>
>EcoR5
GAT ATC CAG CAT GAG AAT GTA ATC GCC AAT CTG CTT ACC CAG TAT GGC TAT ACC AAG
D I Q H E N V I A N L L T Q Y G Y T K>
2200
ATT ATC AAA TGT TAT GAT CCG GGA GTA ACT CCT AAA AAC ATT ATT GAT GCT TTC AAC
I I K C Y D P G V T P K N I I D A F N>
GGA GGA ATC TCG TTG GTC AAC TAT ACG GGC CAC GGT AGC GAA ACA GCT TGG GGT ACG
G G I S L V N Y T G H G S E T A W G T>
2300
TCT CAC TTC GGC ACC ACT CAT GTG AAG CAG CTT ACC AAC AGC AAC CAG CTA CCG TTT
S H F G T T H V K Q L T N S N Q L P F>
>Sph1
2400
ATT TTC GAC GTA GCT TGT GTG AAT GGC GAT TTC CTA TTC AGC ATG CCT TGC TTC GCA
I F D V A C V N G D F L F S M P C F A>
GAA GCC CTG ATG CGT GCA CAA AAA GAT GGT AAG CCG ACA GGT ACT GTT GCT ATC ATA
E A L M R A Q K D G K P T G T V A I I>
2500
CGC TCT ACG ATC AAC CAG TCT TGG GCT TCT CCT ATG CGC GGC CAG GAT GAG ATG AAC
A S T I N Q S W A S P M R G Q D E M N>
GAA ATT CTG TGC GAA AAA CAC CCG AAC AAC ATC AAG CGT ACT TTC GGT GGT GTC ACC
E I L C E K H P N N I K R T F G G V T>
2600
ATG AAC GGT ATG TTT GCT ATG GTG GAA AAG TAT AAA AAG GAT GGT GAG AAG ATG CTC
M N G M F A M V E K Y K K D G E K M L>
GAC ACA TGG ACT GTT TTC GGC GAC CCC TCG CTG CTC GTT CGT ACA CTT GTC CCG ACC
D T W T V F G D P S L L V R T L V P T>
2700
AAA ATG CAG GTT ACG GCT CCG GCT CAG ATT AAT TTG ACG GAT GCT TCA GTC AAC GTA
K M Q V T A P A Q I N L T D A S V N V>
TCT TGC GAT TAT AAT GGT GCT ATT GCT ACC ATT TCA GCC AAT GGA AAG ATG TTC GGT
S C D Y N G A I A T I S A N G K M F G>
>Pst1
2800
TCT GCA GTT GTC GAA AAT GGA ACA GCT ACA ATC AAT CTG ACA GGT CTG ACA AAT GAA
S A V V E N G T A T I N L T G L T N E>
2900

RECTIFIED SHEET (RULE 91)

Table 2 (contd.)

31

AGC ACG CTT ACC CTT ACA GTA GTT GGT TAC AAC AAA GAG ACG GTT ATT AAG ACC ATC
S T L T L T V V G Y N K E T V I K T I>

AAC ACT AAT GST GAG CCT AAC CCC TAC CAG CCC GTT TCC AAC TTG ACA GCT ACA ACG
N T N G E P N P Y Q P V S N L T A T T>

3000

CAG GGT CAG AAA GTA ACG CTC AAG TGG GAT GCA CCG AGC ACG AAA ACC AAT GCA ACC
Q G Q K V T L K W D A P S T K T N A T>

ACT AAT ACC GCT CGC AGC GTG GAT GGC ATA CGA GAA TTG GTT CTT CTG TCA GTC AGC
T N T A R S V D G I R E L V L L S V S>

3100

GAT GCC CCC GAA CTT CTT CGC AGC GGT CAG GCC GAG ATT GTT CTT GAA GCT CAC GAT
D A P E L L R S G Q A E I V L E A H D>

>BamH1

GTT TGG AAT GAT GGA TCC GGT TAT CAG ATT CTT TTG GAT GCA GAC CAT GAT CAA TAT
V W N D G S G Y Q I L L D A D H D Q Y>

3200

GGA CAG GTT ATA CCC AGT GAT ACC CAT ACT CTT TGG CCG AAC TGT AGT GTC CCG GCC
G Q V I P S D T H T L W P N C S V P A>

3300

AAT CTG TTC GCT CCG TTC GAA TAT ACT GTT CCG GAA AAT GCA GAT CCT TCT TGT TCC
N L F A P F E Y T V P E N A D P S C S>

CCT ACC AAT ATG ATA ATG GAT GGT ACT GCA TCC GTT AAT ATA CCG GCC GGA ACT TAT
P T N M I M D G T A S V N I P A G T Y>

3400

GAC TTT GCA ATT GCT GCT CCT CAA GCA AAT GCA AAG ATT TGG ATT GCC GGA CAA GGA
D F A I A A P Q A N A K I W I A G Q G>

CCG ACG AAA GAA GAT GAT TAT GTA TTT GAA GCC GGT AAA AAA TAC CAT TTC CTT ATG
P T K E D D Y V F E A G K K Y H F L M>

3500

AAG AAG ATG GGT AGC GGT GAT GGA ACT GAA TTG ACT ATA AGC GAA GGT GGT GGA AGC
K K M G S G D G T E L T I S E G G G S>

GAT TAC ACC TAT ACT GTC TAT CGT GAC GGC ACG AAG ATC AAG GAA GGT CTG ACG GCT
D Y T Y T V Y R D G T K I K E G L T A>

3600

ACG ACA TTC GAA GAA GAC GGT GTA GCT ACG GGC AAT CAT GAG TAT TGC GTG GAA GTT
T T F E E D G V A T G N H E Y C V E V>

>BamH1

3700

AAG TAC ACA GCC GGC GTA TCT CCG AAG GTA TGT AAA GAC GTT ACG GTA GAA GGA TCC
K Y T A G V S P K V C K D V T V E G S>

AAT GAA TTT GCT CCT GTA CAG AAC CTG ACC GGT AGT GCA GTC GGC CAG AAA GTA ACG

RECTIFIED SHEET (RULE 91)

Table 2 (contd.)

32

N E F A P V Q N L T G S A V G Q K V T>

>Asp718
|
3800

CTC AAG TGG GAT GCA CCT AAT GGT ACC CCG AAT CCA AAT CCG AAT CCG AAT CCG AAT
L K W D A P N G T P N P N P N P N P N>

CCC GGA ACA ACA ACA CTT TCC GAA TCA TTC GAA AAT GGT ATT CCT GCC TCA TGG AAG
P G T T T L S E S F E N G I P A S W K>

>Cla1
|
3900

ACG ATC GAT GCA GAC GGT GAC GGG CAT GGC TGG AAG CCT GGA AAT GCT CCC GGA ATC
T I D A D G D G H G W K P G N A P G I>

GCT GGC TAC AAT AGC AAT GGT TGT GTA TAT TCA GAG TCA TTC GGT CTT GGT GGT ATA
A G Y N S N G C V Y S E S F G L G G I>

4000

GGA GTT CTT ACC CCT GAC AAC TAT CTG ATA ACA CCG GCA TTG GAT TTG CCT AAC GGA
G V L T P D N Y L I T P A L D L P N G>

4100

GGT AAG TTG ACT TTC TGG GTA TGC GCA CAG GAT GCT AAT TAT GCA TCC GAG CAC TAT
G K L T F W V C A Q D A N Y A S E H Y>

GCG GTG TAT GCA TCT TCG ACC GGT AAC GAT GCA TCC AAC TTC ACG AAT GCT TTG TTG
A V Y A S S T G N D A S N F T N A L L>

4200

GAA GAG ACG ATT ACG GCA AAA GGT GTT CGC TCG CCG GAA GCT ATT CGT GGT CGT ATA
E E T I T A K G V R S P E A I R G R I>

CAG GGT ACT TGG CGC CAG AAG ACG GTA GAC CTT CCC GCA GGT ACG AAA TAT GTT GCT
Q G T W R Q K T V D L P A G T K Y V A>

4300

TTC CGT CAC TTC CAA AGC ACG GAT ATG TTC TAC ATC GAC CTT GAT GAG GTT GAG ATC
F R H F Q S T D M F Y I D L D E V E I>

AAG GCC AAC GGC AAG CGC GCA GAC TTC ACG GAA ACG TTC GAG TCT TCT ACT CAT GGA
K A N G K R A D F T E T F E S S T H G>

>Cla1
|
4400

GAG GCA CCG GCG GAA TGG ACT ACT ATC GAT GCC GAT GGC GAT GGT CAG GGT TGG CTC
E A P A E W T T I D A D G D G Q G W L>

4500

TGT CTG TCT TCC GGA CAA TTG GAC TGG CTG ACA GCT CAT GGC GGC ACC AAC GTA GTA
C L S S G Q L D W L T A H G G T N V V>

GCC TCT TTC TCA TGG AAT GGA ATG GCT TTG AAT CCT GAT AAC TAT CTC ATC TCA AAG
A S F S W N G M A L N P D N Y L I S K>

4600

Table 2 (contd.)

33

GAT GTT ACA GGC GCA ACG AAG GTA AAG TAC TAC TAT GCA GTC AAC GAC GGT TTT CCC
D V T G A T K V K Y Y Y A V N D G F P>

GGG GAT CAC TAT GCG GTG ATG ATC TCC AAG ACG GGC ACG AAC GCC GGA GAC TTC ACG
G D H Y A V M I S K T G T N A G D F T>

4700

GTT GTT TTC GAA GAA ACG CCT AAC GGA ATA AAT AAG GGC GGA GCA AGA TTC GGT CTT
V V F E E T P N G I N K G G A R F G L>

TCC ACG GAA GCC AAT GGC GCC AAA CCT CAA AGT GTA TGG ATC GAG CGT ACG GTA GAT
S T E A N G A K P Q S V W I E R T V D>

4800

TTG CCT GCG GGC ACG AAG TAT GTT GCT TTC CGT CAC TAC AAT TGC TCG GAT TTG AAC
L P A G T K Y V A F R H Y N C S D L N>

>Ncol

4900

TAC ATT CTT TTG GAT GAT ATT CAG TTC ACC ATG GGT GGC AGC CCC ACC CCG ACC GAT
Y I L L D D I Q F T M G G S P T P T D>

TAT ACC TAC ACG GTG TAT CGT GAC GGT ACG AAG ATC AAG GAA GGT CTG ACC GAA ACG
Y T Y T V Y R D G T K I K E G L T E T>

5000

ACC TTC GAA GAA GAC GGC GTA GCT ACA GGC AAT CAT GAG TAT TGC GTG GAA GTG AAG
T F E E D G V A T G N H E Y C V E V K>

TAC ACA GCC GGC GTA TCT CCG AAA GAG TGC GTA AAC GTA ACT ATT AAT CCG ACT CAG
Y T A G V S P K E C V N V T I N P T Q>

5100

TTC AAT CCT GTA AAG AAC CTG AAG GCA CAA CCG GAT GGC GGC GAC GTG GTT CTC AAG
F N P V K N L K A Q P D G G D V V L K>

TGG GAA GCC CCG AGC GCA AAA AAG ACA GAA GGT TCT CGT GAA GTA AAA CCG ATC GGA
W E A P S A K K T E G S R E V K R I G>

5200

GAC GGT CTT TTC GTT ACG ATC GAA CCT GCA AAC GAT GTA CGT GCC AAC GAA GCC AAG
D G L F V T I E P A N D V R A N E A K>

5300

GTT GTG CTC GCA GCA GAC AAC GTA TGG GGA GAC AAT ACG GGT TAC CAG TTC TTG TTG
V V L A A D N V W G D N T G Y Q F L L>

GAT GCC GAT CAC AAT ACA TTC GGA AGT GTC ATT CCG GCA ACC GGT CCT CTC TTT ACC
D A D H N T F G S V I P A T G P L F T>

5400

GGA ACA GCT TCT TCC AAT CTT TAC AGT GCG AAC TTC GAG TAT TTG ATC CCG GCC AAT
G T A S S N L Y S A N F E Y L I P A N>

GCC GAT CCT GTT GTT ACT ACA CAG AAT ATT ATC GTT ACA GGA CAG GGT GAA GTT GTA
A D P V V T T Q N I I V T G Q G E V V>

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Table 2 (contd.)

34

5500

ATC CCC GGT GGT GTT TAC GAC TAT TGC ATT ACG AAC CCG GAA CCT GCA TCC GGA AAG
I P G G V Y D Y C I T N P E P A S G K>

ATG TGG ATC GCA GGA GAT GGA GGC AAC CAG CCT GCA CGT TAT GAC GAT TTC ACA TTC
M W I A G D G G N Q P A R Y D D F T F>

5600

GAA GCA GGC AAG AAG TAC ACC TTC ACG ATG CGT CGC GCC GGA ATG GGA GAT GGA ACT
E A G K K Y T F T M R R A G M G D G T>

5700

GAT ATG GAA GTC GAA GAC GAT TCA CCT GCA AGC TAT ACC TAT ACA GTC TAT CGT GAC
D M E V E D D S P A S Y T Y T V Y R D>

GGC ACG AAG ATC AAG GAA GGT CTG ACC GAA ACG ACC TAC CGC GAT GCA GGA ATG AGT
G T K I K E G L T E T T Y R D A G M S>

5800

GCA CAA TCT CAT GAG TAT TGC GTA GAG GTT AAG TAC GCA GCC GGC GTA TCT CCG AAG
A Q S H E Y C V E V K Y A A G V S P K>

GTT TGT GTG GAT TAT ATT CCT GAC GGA GTG GCA GAC GTA ACG GCT CAG AAG CCT TAC
V C V D Y I P D G V A D V T A Q K P Y>

5900

ACG CTG ACA GTT GTT GGA AAG ACG ATC ACG GTA ACT TGC CAA GGC GAA GCT ATG ATC
T L T V V G K T I T V T C Q G E A M I>

TAC GAC ATG AAC GGT CGT CGT CTG GCA GCC GGT CGC AAC ACA GTT GTT TAC ACG GCT
Y D M N G R R L A A G R N T V V Y T A>

6000

CAG GGC GGC TAC TAT GCA GTC ATG GTT GTC GTT GAC GGC AAG TCT TAC GTA GAG AAA
Q G G Y Y A V M V V V D G K S Y V E K>

6100

CTC GCT GTA AAG TAA TTCTGTC TTGGACTCGG AGACTTTGTG CAGACACTTT TAATATAGGT
L A V K >

>Cla1

CTGTAATTGT CTCAGAGTAT GAATCGATCG CCCGACCTCC TTTTAAGGAA GTCTGGGCGA CTTCGTTTTT

6200

ATGCCTATTA TTCTAATATA CTTCTGAAAC AATTTGTTCC AAAAAGTTGC ATGAAAAGAT TATCTTACTA

6300

TCTTTGCACT GCAAAAGGGG AGTTTCCTAA GGTTCCTCCC GGAGTAGTAC GGTAATAACG GTGTGGTAGT

>Pvu2

TCAGCTGGTT AGAATACCTG CCTGTCACGC AGGGGGCTCGG GGGTTCGACT CCCGTCCATA CCGGTAAATA

6400

GCTGAAAGAT AGGCTATAGG TCATCTGAAG CAATTTTAGA AACGAATCCA AAAGCGTCTT AATCCAACG

TABLE 2 (cont'd)

35

```

                                     6500
AATTAAGGCG CTTTTCTTT GTCGCCACCC CACACGTCGG ATGAGGTTCTG GAATAGGCGT ATATTCCGTA
                                     6600
AATATGCCTC CGGTGGTTCC ATTTTGGTTA CAAAAACAA AGGGGCTGAA AATTGTAACC ACAGACGACG
                                     >NdeI
TTAAGACGAT GTTTAGACGA TTGACAAATT ACTCTGTTTC AAAATCATAT GTCGAACTTT GTAGCCGTAT
                                     6700
GGTTACACTA ATTTTGGAGC AAAATGAAGA GTCAATTTCG TTCAGTTTTT TACTTGCGCA GCAATTACAT
                                     6800
CAACAAAGAA GGTAAGACTC CTGTCCTTAT TCGTATTTAT CTGAATAAGG AACGCCTGTC GTTGGGTTCTG
ACAGGGCTGG CTGTTAATCC CATACAATGG GATTTCAGAAA AAGAGAAAGT CAAAGGACAT AGTGCAGAAG
                                     6900
CACTTGAAGT CAATCGAAAG ATCGAAGAAA TCAGGGCTGA TATTCTGACC ATTTACAAAC GTTTGGAAGT
                                     7000
AACAGTAGAT GATTTGACGC CGGAGAGGAT CAAATCGGAA TACTGCGGAC AGACGGATAC ATTAACAGT
ATAGTGGAAC TTTTCGATAA ACATAACGAG GATGTCCGGG CCCAGGTGGG AATCAATAAA ACGGCTGCCA
                                     7100
CTTTACAAA ATACGAAAAC AGCAAACGGC ATTTTACCCG ATTCCTCAA GCGAAGTACA ACAGAACGGA
                                     7200
TCTCAAATTC TCAGAGCTTA CCCCGTTGGT CATTTCATAAC TTTGAGATAT ATCTGCTGAC TGTAGCCCAT
                                     >Hind3
TGTTGCCCCG ATACGGCAAC CAAAATCTTG AAGCTT
```

Cleavage of the precursor protein after the Arg residue at amino acid 227 removes the N-terminal precursor portion and after the Arg residue at amino acid 719, 1091 and 1429 releases a low molecular weight Arg-gingipain and three hemagglutinin components. The 44 kDa hemagglutinin component has an amino acid sequence as given in SEQ ID NO:11 from 720-1091, with calculated molecular weight of 39.4 kDa, consistent with that estimated by gel electrophoresis. The 17 kDa hemagglutinin component has an amino acid sequence as given in SEQ ID NO:11 at amino acids 1092-1429, and a calculated molecular weight of 37.1 kDa. The 27 kDa hemagglutinin component has an amino acid sequence extending from amino acids 1430-1704 in SEQ ID NO:11, and a calculated molecular weight of 29.6 kDa.

TABLE 3
Alignment of Hemagglutinin Domain Sequences shown in Fig.2.

RGPI	amino acids 670-674 of SEQ ID NO:11	Ltatt
HGP-44kDa ¹	amino acids 865-913 of SEQ ID NO:11	dYTYTVYRDGKIKEGLTaTTfeedGvatgnHEYCVVEVKYtAGVSPKvC
HGP-17kDa	amino acids 1320-1368 of SEQ ID NO:11	dYTYTVYRDGKIKEGLTeTTfeedGvatgnHEYCVVEVKYtAGVSPKeC
HGP-27kDa	amino acids 1580-1626 of SEQ ID NO:11	sYTYTVYRDGKIKEGLTeTTyrdaGmsaqsHEYCVVEVKYaAGVSPKvC
1 RGP means Arg-gingipain proteolytic component		
2 HGP means Hemagglutinin protein component		

Table 3 is the result of sequence comparison of the 44 kDa, 27 kDa and 17 kDa hemagglutinin domains of Arg-gingipain complexes, alignment of regions of amino acid identity, which without wishing to be bound by any particular theory, are postulated to be the domains responsible for hemagglutinin activity. Identical amino acids among all hemagglutinin domains are in capital letters, and amino acids which are not conserved are shown in lower case letters. In the case of the proteolytic component, only a limited region with significant match is shown.

A genomic DNA library was also prepared from virulent P. gingivalis W50. Two clones were identified as containing Arg-gingipain coding sequence. 0.5 and 3.5 kb *Bam*HI fragments were sequenced; it exhibited 99% nucleotide sequence identity with about 3160 plus 557 bp of P. gingivalis H66 DNA containing Arg-gingipain coding sequence. A comparison of the deduced amino acid sequences of the encoded Arg-gingipain sequences revealed 99% identity.

Tables 1 and 2 both represent sequences from P. gingivalis. However, it is understood that there will be some variations in the amino acid sequences and encoding nucleic acid sequences for Arg-gingipain from different P. gingivalis strains. The ordinary skilled artisan can readily identify and isolate Arg-gingipain-encoding sequences from other strains where there is at least 70% homology to the specifically exemplified sequences herein using the sequences provided herein taken with what is well known to the art. Also within the scope of the present invention are Arg-gingipain where the protease or proteolytic component has at least about 85% amino acid sequence identity with an amino acid sequence exemplified herein.

It is also understood by the skilled artisan that there can be limited numbers of amino acid substitutions in a protein without significantly affecting function, and that nonexemplified gingipain-1 proteins can have some amino acid sequence diversion from the exemplified amino acid sequence. Such naturally

occurring variants can be identified, e.g., by hybridization to the exemplified (mature) Arg-gingipain-2 coding sequence (or a portion thereof capable of specific hybridization to Arg-gingipain sequences) under conditions appropriate to detect at least about 70% nucleotide sequence homology, preferably about 80%, more preferably about 90% and most preferably 95-100% sequence homology. Preferably the encoded Arg-gingipain protease or proteolytic component has at least about 85% amino acid sequence identity to an exemplified Arg-gingipain amino acid sequence.

It is well known in the biological arts that certain amino acid substitutions can be made in protein sequences without affecting the function of the protein. Generally, conservative amino acids are tolerated without affecting protein function. Similar amino acids can be those that are similar in size and/or charge properties, for example, aspartate and glutamate and isoleucine and valine are both pairs of similar amino acids. Similarity between amino acid pairs has been assessed in the art in a number of ways. For example, Dayhoff et al. (1978) in *Atlas of Protein Sequence and Structure*, Volume 5, Supplement 3, Chapter 22, pages 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed as a measure of amino acid similarity. Dayhoff et al.'s frequency tables are based on comparisons of amino acid sequences for proteins having the same function from a variety of evolutionarily different sources.

The skilled artisan recognizes that other P. gingivalis strains can have coding sequences for a protein with the distinguishing characteristics of an Arg-gingipain; those coding sequences may be identical to or synonymous with the exemplified coding sequence, or there may be some variation(s) in the encoded amino acid sequence. An Arg-gingipain coding sequence from a P. gingivalis strain other than H66 can be identified by, e.g. hybridization to a polynucleotide or an oligonucleotide having the whole or a portion of the exemplified coding sequence for

mature gingipain, under stringency conditions appropriate to detect a sequence of at least 70% homology.

5 A polynucleotide or fragment thereof is "substantially homologous" (or "substantially similar") to another polynucleotide if, when optimally aligned (with appropriate nucleotide insertions or deletions) with another polynucleotide, there is nucleotide sequence identity for approximately 60% of the nucleotide bases, usually approximately 70%, more usually
10 about 80%, preferably about 90%, and more preferably about 95% to 100% of the nucleotide bases.

Alternatively, substantial homology (or similarity) exists when a polynucleotide or fragment thereof will hybridize to
15 another under polynucleotide under selective hybridization conditions. Selectivity of hybridization exists under hybridization conditions which allow one to distinguish the target polynucleotide of interest from other polynucleotides. Typically, selective hybridization will occur when there is
20 approximately 55% similarity over a stretch of about 14 nucleotides, preferably approximately 65%, more preferably approximately 75%, and most preferably approximately 90%. See Kanehisa (1984) *Nuc. Acids Res.*, 12:203-213. The length of homology comparison, as described, may be over longer stretches,
25 and in certain embodiments will often be over a stretch of about 17 to 20 nucleotides, and preferably about 36 or more nucleotides.

The hybridization of polynucleotides is affected by such
30 conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing polynucleotides, as will be readily appreciated by those skilled in the art. Stringent
35 temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be

less than 1 M, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter (Wetmur and Davidson (1968) *J. Mol. Biol.* 31, 349-370).

5

An "isolated" or "substantially pure" polynucleotide is a polynucleotide which is substantially separated from other polynucleotide sequences which naturally accompany a native gingipain-1 sequence. The term embraces a polynucleotide
10 sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, chemically synthesized analogues and analogues biologically synthesized by heterologous systems.

15

A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide of a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the
20 sequence.

25

A nucleotide sequence is operably linked when it is placed into a functional relationship with another nucleotide sequence. For instance, a promoter is operably linked to a coding sequence
25 if the promoter affects its transcription or expression. Generally, operably linked means that the sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, it is well known that certain genetic elements, such as enhancers, may be
30 operably linked even at a distance, i.e., even if not contiguous.

35

The term "recombinant" polynucleotide refers to a polynucleotide which is made by the combination of two otherwise separated segments of sequence accomplished by the artificial manipulation of isolated segments of polynucleotides by genetic engineering techniques or by chemical synthesis. In so doing one

may join together polynucleotide segments of desired functions to generate a desired combination of functions.

5 Polynucleotide probes include an isolated polynucleotide attached to a label or reporter molecule and may be used to identify and isolate other Arg-gingipain coding sequences. Probes comprising synthetic oligonucleotides or other polynucleotides may be derived from naturally occurring or recombinant single or double stranded nucleic acids or be
10 chemically synthesized. Polynucleotide probes may be labelled by any of the methods known in the art, e.g., random hexamer labeling, nick translation, or the Klenow fill-in reaction.

15 Large amounts of the polynucleotides may be produced by replication in a suitable host cell. Natural or synthetic DNA fragments coding for a proteinase or a fragment thereof will be incorporated into recombinant polynucleotide constructs, typically DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the
20 construct will be suitable for replication in a unicellular host, such as yeast or bacteria, but a multicellular eukaryotic host may also be appropriate, with or without integration within the genome of the host cells. Commonly used prokaryotic hosts include strains of Escherichia coli, although other prokaryotes,
25 such as Bacillus subtilis or Pseudomonas may also be used. Mammalian or other eukaryotic host cells include yeast, filamentous fungi, plant, insect, amphibian and avian species. Such factors as ease of manipulation, ability to appropriately glycosylate expressed proteins, degree and control of protein
30 expression, ease of purification of expressed proteins away from cellular contaminants or other factors may determine the choice of the host cell.

35 The polynucleotides may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) *Tetra. Letts.*, 22: 1859-1862 or the triester method according to Matteucci et al. (1981) *J. Am. Chem.*

Soc., 103: 3185, and may be performed on commercial automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

DNA constructs prepared for introduction into a prokaryotic or eukaryotic host will typically comprise a replication system (i.e. vector) recognized by the host, including the intended DNA fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide-encoding segment. Expression systems (expression vectors) may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Signal peptides may also be included where appropriate from secreted polypeptides of the same or related species, which allow the protein to cross and/or lodge in cell membranes or be secreted from the cell.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al. (1989) vide infra; Ausubel et al. (Eds.) (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York; and Metzger et al. (1988) Nature, 334: 31-36. Many useful vectors for expression in bacteria, yeast, mammalian, insect, plant or other cells are well known in the art and may be obtained such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made.

For appropriate enhancer and other expression control sequences, see also Enhancers and Eukaryotic Gene Expression, Cold Spring Harbor Press, N.Y. (1983). While such expression vectors may replicate autonomously, they may less preferably replicate by
5 being inserted into the genome of the host cell.

Expression and cloning vectors will likely contain a selectable marker, that is, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the
10 vector. Although such a marker gene may be carried on another polynucleotide sequence co-introduced into the host cell, it is most often contained on the cloning vector. Only those host cells into which the marker gene has been introduced will survive and/or grow under selective conditions. Typical selection genes
15 encode proteins that (a) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper selectable marker will depend on the host cell;
20 appropriate markers for different hosts are known in the art.

The recombinant vectors containing the Arg-gingipain coding sequences of interest can be introduced (transformed, transfected) into the host cell by any of a number of appropriate
25 means, including electroporation; transformation or transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and transfection or infection (where the vector is an infectious agent, such as a viral or retroviral genome). The
30 choice of such means will often depend on the host cell. Large quantities of the polynucleotides and polypeptides of the present invention may be prepared by transforming suitable prokaryotic or eukaryotic host cells with gingipain-1-encoding polynucleotides of the present invention in compatible vectors
35 or other expression vehicles and culturing such transformed host cells under conditions suitable to attain expression of the Arg-

gingipain-encoding gene. The Arg-gingipain may then be recovered from the host cell and purified.

5 The coding sequence for the "mature" form of Arg-gingipain-2 is expressed after PCR site-directed mutagenesis and cloning into an expression vector suitable for use in E. coli, for example. Exemplary expression vectors for E. coli and other host cells are given, for example in Sambrook et al. (1989), vide infra, and in Pouwels et al. (Eds.) (1986) Cloning Vectors, Elsevier Science
10 Publishers, Amsterdam, the Netherlands.

In order to eliminate leader sequences and precursor sequences at the 5' side of the coding sequence, a combination of restriction endonuclease cutting and site-directed mutagenesis
15 via PCR using an oligonucleotide containing a desired restriction site for cloning (one not present in coding sequence), a ribosome binding site, an translation initiation codon (ATG) and the codons for the first amino acids of the mature Arg-gingipain-2. The oligonucleotide for site-directed mutagenesis at the 3' end
20 of the coding sequence for mature gingipain-1 includes nucleotides encoding the carboxyterminal amino acids of mature gingipain-1, a translation termination codon (TAA, TGA or TAG), and a second suitable restriction endonuclease recognition site not present in the remainder of the DNA sequence to be inserted
25 into the expression vector. The site-directed mutagenesis strategy is similar to that of Boone et al. (1990) *Proc. Natl. Acad. Sci. USA* 87: 2800-2804, as modified for use with PCR.

In another embodiment, polyclonal and/or monoclonal
30 antibodies capable of specifically binding to a proteinase or fragments thereof are provided. The term antibody is used to refer both to a homogenous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Monoclonal or polyclonal antibodies specifically
35 reacting with the Arg-gingipains may be made by methods known in the art. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratories; Goding (1986)

Monoclonal Antibodies: Principles and Practice, 2d ed., Academic Press, New York; and Ausubel et al. (1987) supra. Also, recombinant immunoglobulins may be produced by methods known in the art, including but not limited to the methods described in U.S. Patent No. 4,816,567. Monoclonal antibodies with affinities of $10^8 M^{-1}$, preferably 10^9 to 10^{10} or more are preferred.

Antibodies specific for Arg-gingipains may be useful, for example, as probes for screening DNA expression libraries or for detecting the presence of Arg-gingipains in a test sample. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or noncovalently, a substance which provides a detectable signal. Suitable labels include but are not limited to radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. United States Patents describing the use of such labels include but are not limited to Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

Antibodies specific for Arg-gingipain(s) and capable of inhibiting its proteinase activity may be useful in treating animals, including man, suffering from periodontal disease. Such antibodies can be obtained by the methods described above and subsequently screening the Arg-gingipain-specific antibodies for their ability to inhibit proteinase activity.

Compositions and immunogenic preparations including vaccine compositions comprising substantially purified recombinant Arg-gingipain(s) and a suitable carrier therefor are provided. Alternatively, hydrophilic regions of the proteolytic component or hemagglutinin component(s) of Arg-gingipain can be identified by the skilled artisan, and peptide antigens can be synthesized and conjugated to a suitable carrier protein (e.g., bovine serum albumin or keyhole limpet hemocyanin) for use in vaccines or in raising antibody specific for Arg-gingipains. Immunogenic compositions are those which result in specific antibody

production when injected into a human or an animal. Such vaccines are useful, for example, in immunizing an animal, including humans, against inflammatory response and tissue damage caused by P. gingivalis in periodontal disease. The vaccine preparations comprise an immunogenic amount of one or more Arg-gingipains or an immunogenic fragment(s) or subunit(s) thereof. Such vaccines may comprise one or more Arg-gingipain proteinases, or in combination with another protein or other immunogen. By "immunogenic amount" is meant an amount capable of eliciting the production of antibodies directed against Arg-gingipain(s) in an individual to which the vaccine has been administered.

Immunogenic carriers may be used to enhance the immunogenicity of the proteinases. Such carriers include but are not limited to proteins and polysaccharides, liposomes, and bacterial cells and membranes. Protein carriers may be joined to the proteinases to form fusion proteins by recombinant or synthetic means or by chemical coupling. Useful carriers and means of coupling such carriers to polypeptide antigens are known in the art.

The vaccines may be formulated by any of the means known in the art. Such vaccines are typically prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also, for example, be emulsified, or the protein encapsulated in liposomes.

The active immunogenic ingredients are often mixed with excipients or carriers which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. The concentration of the immunogenic polypeptide in injectable formulations is usually in the range of 0.2 to 5 mg/ml.

In addition, if desired, the vaccines may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide; N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP); N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE); and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogen resulting from administration of the immunogen in vaccines which are also comprised of the various adjuvants. Such additional formulations and modes of administration as are known in the art may also be used.

50 kDa Arg-gingipain or high molecular weight Arg-gingipain and fragments thereof may be formulated into vaccines as neutral or salt forms. Pharmaceutically acceptable salts include but are not limited to the acid addition salts (formed with free amino groups of the peptide) which are formed with inorganic acids, e.g., hydrochloric acid or phosphoric acids; and organic acids, e.g., acetic, oxalic, tartaric, or maleic acid. Salts formed with the free carboxyl groups may also be derived from inorganic bases, e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides, and organic bases, e.g., isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine, and procaine.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of about 100 to 1,000 μg of protein per dose, more generally in the range of about 5 to 500 μg of protein per dose, depends on the subject to

be treated, the capacity of the individual's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of the active ingredient required to be administered may depend on the judgment of the physician or
5 doctor of dental medicine and may be peculiar to each individual, but such a determination is within the skill of such a practitioner.

The vaccine or other immunogenic composition may be given
10 in a single dose or multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may include 1 to 10 or more separate doses, followed by other doses administered at subsequent time intervals as required to maintain and or reinforce the immune response, e.g., at 1 to 4 months for
15 a second dose, and if needed, a subsequent dose(s) after several months.

Recombinant Arg-gingipains are useful in methods of identifying agents that modulate proteinase activity, e.g., by
20 acting on the proteinase itself. One such method comprises the steps of incubating Arg-gingipain-1 (or high molecular weight Arg-proteinase) with a putative therapeutic agent; determining the activity of the proteinase incubated with the agent; and comparing the activity obtained in step with the activity of a
25 control sample of proteinase that has not been incubated with the agent.

All references cited herein are hereby incorporated by reference in their entirety.

30 Except as noted hereafter, standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are
35 those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor

Laboratory, Plainview, New York; Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* 218, Part I; Wu (ed.) (1979) *Meth Enzymol.* 68; Wu et al. (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold spring Harbor Laboratory, Cold Spring Harbor, New York, Old Primrose (1981) Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink (1982) Practical Methods in Molecular Biology; Glover (ed.) (1985) DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; Setlow and Hollaender (1979) Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

The foregoing discussion and the following examples illustrate but are not intended to limit the invention. The skilled artisan will understand that alternative methods may be used to implement the invention.

EXAMPLES

Example 1 Purification of Gingipain Enzymes

Example 1.1 Bacterial Cultivation

P. gingivalis strains H66 (ATCC 33277) and W50 (ATCC 53978) (virulent) were used in these studies. Cells were grown in 500 ml of broth containing 15.0 g Trypticase Soy Broth (Difco, Detroit, Michigan), 2.5 g yeast extract, 2.5 mg hemin, 0.25 g cysteine, 0.05 g dithiothreitol, 0.5 mg menadione (all from Sigma Chemical Company, St. Louis, MO) anaerobically at 37°C for 48 hr in an atmosphere of 85% N₂, 10% CO₂, 5% H₂. The entire 500 ml culture was used to inoculate 20 liters of the same medium, and the latter was incubated in a fermentation tank at 37°C for 48 hr (to a final optical density of 1.8 at 650 nm).

Example 1.2 Purification of Low Molecular Weight Arg-gingipain

1200 ml cell-free supernatant was obtained from the 48 hr culture by centrifugation at 18,000 x g for 30 min. at 4°C. Proteins in the supernatant were precipitated out by 90% saturation with ammonium sulfate. After 2 hr at 4°C, the suspension was centrifuged at 18,000 x g for 30 min. The resulting pellet was dissolved in 0.05 M sodium acetate buffer, pH 4.5, 0.15 NaCl, 5 mM CaCl₂; the solution was dialyzed against the same buffer overnight at 4°C, with three changes with a buffer:protein solution larger than 150:1. The dialysate was then centrifuged at 25,000 x g for 30 min., and the dark brown supernatant (26 ml) was then chromatographed over an agarose gel filtration column (5.0 x 150 cm; Sephadex G-150, Pharmacia, Piscataway, NJ) which had been pre-equilibrated with the same buffer. The column was developed with said buffer at a flow rate of 36 ml/hr. 6 ml fractions were collected and assayed for both amidolytic and proteolytic activities, using Bz-L-Arg-pNA and azocasein as substrates. Four peaks containing amidolytic activity were identified (Fig. 1). The fractions corresponding to peak 4 were combined, concentrated by ultrafiltration (Amicon PM-10 membrane; Amicon, Beverly, MA) and then dialyzed overnight against 0.05 Bis-Tris, 5 mM CaCl₂, pH 6.0. The volume of the dialysate was 14 ml.

The 14 ml dialysate from the previous step was then applied to a DEAE-cellulose (Whatman, Maidstone, England) column (1 x 10 cm) equilibrated with 0.05 mM Bis-Tris, 5 mM CaCl₂, pH 6.0. The column was then washed with an additional 100 ml of the same buffer. About 75% of the amidolytic activity, but only about 50% of the protein, passed through the column. The column wash fluid was dialyzed against 0.05 M sodium acetate buffer containing 5 mM CaCl₂ (pH 4.5). This 19 ml dialysate was applied to a Mono S FPLC column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) equilibrated with the same buffer. The column was washed with the starting buffer at a flow rate of 1.0 ml/min for 20 min. Bound proteins were eluted first with a linear NaCl gradient (0 to 0.1 M) followed by a second linear NaCl gradient (0.1 to 0.25

M), each gradient applied over a 25 min time period. Fractions were assayed for amidolytic activity using Bz-L-Arg-pNA. Fractions with activity were pooled and re-chromatographed using the same conditions. Although not detectable by gel electrophoresis, trace contamination by a proteinase capable of cleaving after lysyl residues was sometimes observed. This contaminating activity was readily removed by applying the sample to an arginyl-agarose column (L-Arginyl-SEPHAROSE 4B) equilibrated with 0.025 M Tris-HCl, 5 mM CaCl₂, 0.15 M NaCl, pH 7.5. After washing with the same buffer, purified enzyme was eluted with 0.05 M sodium acetate buffer, 5 mM CaCl₂, pH 4.5. Yields of gingipain-1 were markedly reduced by this step (about 60%).

Example 1.3 High Molecular Weight Arg-gingipain Purification

The culture supernatant (2,900 ml) was obtained by centrifugation of the whole culture (6,000 x g, 30 min, 4°C). Chilled acetone (4,350 ml) was added to this fraction over a period of 15 min, with the temperature of the solution maintained below 0°C at all times, using an ice/salt bath and this mixture was centrifuged (6,000 x g, 30 min, -15°C). The precipitate was dissolved in 290 ml of 20 mM Bis-Tris-HCl, 150 mM NaCl, 5 mM CaCl₂, 0.02% (w/v) NaN₃, pH 6.8 (Buffer A), and dialyzed against Buffer A containing 1.5 mM 4,4'-Dithiodipyridine disulfide for 4h, followed by 2 changes of buffer A overnight. The dialyzed fraction was centrifuged (27,000 x g, 30 min, 4°C), following which it was concentrated to 40 ml by ultrafiltration using an Amicon PM-10 membrane. This concentrated fraction was applied to a Sephadex G-150 column (5 x 115 cm = 2260 ml; Pharmacia, Piscataway, NJ) which had previously been equilibrated with Buffer A, and the fractionation was carried out at 30 ml/h (1.5 cm/h). Fractions (9 ml) were assayed for activity against Bz-L-Arg-pNa and Z-L-Lys-pNa (Novabiochem; 0.5 mM). Amidolytic activities for Bz-L-Arg-pNa (0.5 mM) or Z-L-Lys-pNa were measured in 0.2 M Tris.HCl, 1 mM CaCl₂, 0.02% (w/v) NaN₃, 10 mM L-cysteine, pH 7.6. General proteolytic activity was measured with azocasein (2% w/v) as described by Barrett and Kirschke (1981) Meth.

Enzymol. 80, 535-561 for cathepsin L. Three peaks with activity against the two substrates were found. The first (highest molecular weight) peak of activity was pooled, concentrated to 60 ml using ultrafiltration and dialyzed overnight against two changes of 50 mM Tris-HCl, 1 mM CaCl₂, 0.02% NaN₃, pH 7.4 (Buffer B).

This high MW fraction was applied to an L-Arginine-Sepharose column (1.5 x 30 cm = 50 ml), which had previously been equilibrated with Buffer B at a flow rate of 20 ml/hr (11.3 cm/h), following which the column was washed with two column volumes of Buffer B. Following this, a step gradient of 500 mM NaCl was applied in Buffer B and the column was washed with this concentration of NaCl until the A₂₈₀ baseline fell to zero. After re-equilibration of the column in Buffer B, a gradient from 0-750 mM L-Lysine was applied in a total volume of 300 ml, followed by 100 ml of 750 mM L-Lysine. The column was once again re-equilibrated with Buffer B and a further gradient to 100 mM L-arginine in 300 ml was applied in the same way. Fractions (6 ml) from the Arg wash were assayed for activity against the two substrates as described previously. The arginine gradient eluted a major peak for an enzyme degrading Bz-L-Arg-pNa. The active fractions were pooled and dialyzed against two changes of 20 mM Bis-Tris-HCl, 1 mM CaCl₂, 0.02% (v/w) NaN₃, pH 6.4 (Buffer C) and concentrated down to 10 ml using an Amicon PM-10 membrane.

The concentrate with activity for cleaving Bz-L-Arg-pNa was applied to a Mono Q FPLC column (Pharmacia LKB Biotechnology Inc, Piscataway, NJ) equilibrated in Buffer C, the column was washed with 5 column volumes of Buffer C at 1.0 ml/min, following which bound protein was eluted with a 3 step gradient [0-200 mM NaCl (10 min), followed by 200-250 mM NaCl (15 min) and 250-500 mM NaCl (5 min)]. The active fractions from Mono Q were pooled and used for further analyses.

Example 2 Molecular Weight Determination

The molecular weight of the purified Arg-gingipain-1 was estimated by gel filtration on a Superose 12 column (Pharmacia, Piscataway, NJ) and by Tricine-SDS polyacrylamide gel electrophoresis. In the latter case, 1 mM TLCK was used to inactivate the protease prior to boiling, thus preventing autoprolytic digestion.

Example 3 Enzyme Assays

Amidolytic activities of *P. gingivalis* proteinases were measured with the substrates MeO-Suc-Ala-Ala-Pro-Val-pNA at a concentration of 0.5 mM, Suc-Ala-Ala-Ala-pNA (0.5 mM), Suc-Ala-Ala-Pro-Phe-pNA (0.5 mM), Bz-Arg-pNA (1.0 mM), Cbz-Phe-Leu-Glu-pNA (0.2 mM); S-2238, S-2222, S-2288 and S-2251 each at a concentration of 0.05 mM; in 1.0 ml of 0.2 M Tris-HCl, 5mM CaCl₂, pH 7.5. In some cases either 5 mM cysteine and/or 50 mM glycyl-glycine (Gly-Gly) was also added to the reaction mixture.

For routine assays, pH optimum determination and measurement of the effect of stimulating agents and inhibitors on trypsin-like enzymes, only Bz-L-Arg-pNA was used as substrate. Potential inhibitory or stimulatory compounds were preincubated with enzyme for up to 20 min at room temperature at pH 7.5, in the presence of 5 mM CaCl₂ (except when testing the effects of chelating agents) prior to the assay for enzyme activity.

General proteolytic activity was assayed using the same buffer system as described for detecting amidolytic activity, but using azocoll or azocasein (1% w/v) as substrate.

A unit of Arg-gingipain-1 enzymatic activity is based on the spectroscopic assay using benzoyl-Arg-p-nitroanilide as substrate and recording Δ absorbance units at 405 nm/min/absorbance unit at 280 nm according to the method of Chen et al. (1992) supra.

Example 4 Enzyme Specificity

Purified Arg-gingipain-1 (0.8 μ g) in 50 mM ammonium bicarbonate buffer, pH 7.7, 5 mM CaCl_2 , was preincubated with 2 mM cysteine for 10 min, followed by the addition of either oxidized insulin B chain (225 μ g) or melittin (225 μ g) at 25°C. Samples were removed after various time intervals, and the reaction mixtures were subjected to HPLC (reverse phase column, MicroPak SP C-18 column) using linear gradients (0.08% trifluoroacetic acid to 0.08% trifluoroacetic acid plus 80% acetonitrile, over a 45 min period (flow rate 1.0 ml/min). Peptides were detected by monitoring A_{220} . Product peaks were collected and subjected to amino acid analysis and/or amino-terminal sequence analysis.

Example 5 Amino Acid Sequence Analysis

Amino-terminal amino acid sequence analysis of either Arg-gingipain-1 or degradation products from proteolytic reactions was carried out using an Applied Biosystems 4760A gas-phase sequenator, using the program designed by the manufacturer.

The amino acid sequence of the COOH terminus of SDS-denatured Arg-gingipain-1 and of Arg-gingipain-2 was determined. 10 nmol aliquots of gingipain-1 were digested in 0.2 M N-ethylmorpholine acetate buffer, pH 8.0, with carboxypeptidase A and B at room temperature, using 1:100 and 1:50 molar ratios, respectively. Samples were removed at intervals spanning 0 to 12 hours, boiled to inactivate the carboxypeptidase, and protein was precipitated with 20% trichloroacetic acid. Amino acid analysis was performed on the supernatants.

Example 6 Materials

MeO-Suc-Ala-Ala-Pro-Val-pNA, Suc-Ala-Ala-Pro-Phe-pNA, Gly-Pro-pNA, Suc-Ala-Ala-Ala-pNA, Bz-Arg-pNA, diisopropylfluorophosphate, phenylmethanesulfonyl fluoride, tosyl-L-lysine chloromethyl ketone (TLCK), tosyl-L-phenylalanine chloromethyl ketone (TPCK), trans-epoxysuccinyl-L-leucylamide-(4-guanidino)butane), an inhibitor of cysteine proteinases,

leupeptin, antipain and azocasein were obtained from Sigma Chemical Co., St. Louis, MO. 3,4-Dichloroisocoumarin was obtained from Boehringer, Indianapolis, IN and CBz-Phe-Leu-Glu-pNA and azocoll were obtained from Calbiochem, La Jolla, CA. S-2238 (D-Phe-Pip-Arg-pNA), S-2222 (Bz-Ile-Glu-(γ -OR)-Gly-Arg-pNA), S-2288 (D-Ile-Pro-Arg-pNA), and S-2251 (D-Val-Leu-Lys-pNA) were from Kabi-Vitrum, (Beaumont, Texas).

Example 7 Electrophoresis

SDS-PAGE of Arg-gingipain-1 was performed as in Laemmli (1970) *Nature* 227: 680-685. Prior to electrophoresis the samples were boiled in a buffer containing 20% glycerol, 4% SDS, and 0.1% bromphenol blue. The samples were run under reducing conditions by adding 2% β -mercaptoethanol unless otherwise noted. Samples were heated for 5 min at 100°C prior to loading onto gels. A 5-15% gradient gel was used for the initial digests of C3 and C5, and the gels were subsequently stained with Coomassie Brilliant Blue R. The C5 digest used to visualize breakdown products before and after reduction of the disulfide bonds were electrophoresed in a 8% gel. Attempts to visualize C5a in the C5 digest were carried out using 13% gels that were developed with silver stain according to the method of Merril et al. (1979) *Proc. Natl. Acad. Sci USA* 76, 4335-4340.

In some experiments (high molecular weight forms) SDS-PAGE using Tris-HCl/Tricine buffer was carried out per Shagger and Van Jagow (1987) *Analyt. Biochem.* 166, 368-379.

Electrophoresis on cellulose acetate strips were performed in 0.075 barbital buffer at pH 8.5 and 4°C for 30 min. at 200 V. The Beckman Microzone apparatus (model R101) used for the electrophoresis of the protein, and the strips were stained using Amido Black.

Example 8 Oligonucleotide Synthesis

Oligonucleotide primers for PCR probes and sequencing were synthesized by the phosphoraminitite method with an Applied

Biosystems model 394 automated DNA synthesizer (Applied Biosystems, Foster City, CA) and purified by PAGE and desalted on Sep-Pak (Millipore Corp., Beverly, MA) using standard protocols. Primer GIN-1-32 was designed to bind to the noncoding strand of Arg-gingipain DNA corresponding to the NH₂-terminal portion of the mature protein, i.e., to the sequence encoding amino acids 2-8 within SEQ ID NO:1. The sequence of the 32-base primer consists of 20 bases specific for Arg-gingipain and six additional bases at the 5' end (underlined), as follows: 5'-GGCTTTACNCCNGTNGARGARYTNGA-3' (SEQ ID NO:6), where N is A or G or C or T. Primer GIN-2-30 was designed to bind to the coding strand of Arg-gingipain DNA corresponding to the amino acids 25-32 of the mature protein, i.e., residues 25-32 of SEQ ID NO:1. The sequence of the 30-base primer consists of 24 bases specific for gingipain-1 (and gingipain-2) DNA and six additional bases at the 5' end (underlined), as follows: 5'-GGCTTTRTTYTTCCARTCNACRAARTCYTT-3', where R is A or G, Y is C or T and N is A or G or C or T (SEQ ID NO:7). Primer GIN-8S-48: 5'-CCTGGAGAATTCTCGTATGATCGTCATCGTAGCCAAAAAGTATGAGGG-3' (SEQ ID NO:8) was designed to bind to the noncoding strand of Arg-gingipain DNA corresponding to the amino acids 11-22 of the mature protein, i.e., amino acids 11-22 of SEQ ID NO:1, and was designed on the basis of partial DNA sequence information for the Arg-gingipain coding sequence (nucleotides 1659-1694 of SEQ ID NO:3) and included a 6-base *EcoRI* restriction site plus six additional bases at the 5' end (underlined). This primer was used as a probe to screen a λ DASH *P. gingivalis* genomic DNA library (see below). One additional oligonucleotide GIN-14-20 (20-mers), initially designed to sequence Arg-gingipain DNA, was used as a probe to identify and then clone the 3' end of the gingipain-1 coding sequence, as a *Pst*I-*Hind*III sequence. Primer GIN-14-20 was designed to bind to the noncoding strand of gingipain-1 DNA corresponding to 20 bases specific for 3' end of Arg-gingipain (nucleotides 2911-2930 within SEQ ID NO:3): 5'-ATCAACACTAATGGTGAGCC-3' (SEQ ID NO:9). A total of 71 20-mers internal primers were designed using empirically determined sequence to sequence the Arg-gingipain locus.

Example 9 Polymerase Chain Reaction

The DNA templates used in PCR was P. gingivalis strain H66 total cellular DNA. The PCR was run using primer GIN-1-32 (SEQ ID NO:6) along with primer GIN-2-30 (SEQ ID NO:7); PCR consistently yielded a single 105-base pair product (P105) detected on a 7% acrylamide gel representing a partial gingipain DNA. After treatment with the Klenow enzyme, P105 was cloned in pCR-ScriptTMSK(+) (Stratagene La Jolla, CA). After sequence analysis of P105, specific primer GIN-8S-48 (SEQ ID NO:8) was designed to use as a probe. The ³²P-labeled GIN-8S-48 probe, was generated by kinase reaction for use in subsequent hybridization screening of the λ DASH library. Incorporated nucleotides were separated from unincorporated nucleotides on a Sephadex G-25 column (Boehringer Mannheim Corporation, Indianapolis, IN).

Example 10 Construction and Screening of the genomic DNA library

λ DASH and λ ZAP DNA libraries were constructed according to the protocols of Stratagene, using the lambda DASHTM II/BamHI cloning kit and DNA preparations from P. gingivalis strains H66 and W50. Libraries of 3×10^5 independent recombinant clones was obtained using P. gingivalis H66 DNA, and 1.5×10^5 independent recombinant clones were obtained from P. gingivalis W50 DNA.

Approximately 3×10^5 phages were grown on 5x150 mm agar plates, lifted in duplicate onto supported nitrocellulose transfer membrane (BAS-NC, Schleicher & Schuell, Keene, NH), hybridized to the ³²P-labeled GIN-8S-48 probe described above. Hybridizations were performed overnight at 42°C in 2X Denhardt's solution (Denhardt, D.T. (1966), *Biochem. Biophys. Res. Comm.* **23**, 641-646), 6X SSC (SSC is 15 mM sodium citrate, 150 mM NaCl), 0.4% SDS (w/v), 500 μ g/ml fish sperm DNA. The filters were washed in 2X SSC containing 0.05% SDS (w/v) at 48°C. Seven positively hybridizing plaques were purified. After extraction and purification, the DNA was analyzed by restriction enzyme digestion and agarose gel electrophoresis. The 3 kb-*Pst*I fragment from clone A1 (P. gingivalis H66) was subsequently cloned into pBluescript SK(-) (Stratagene, La Jolla, CA) and

M13mp18 and 19 and sequenced. After restriction analysis of the A1 clone, a *Sma*I/*Bam*HI fragment was then cloned into pBluescript SK(-). A *Pst*I/*Bam*HI smaller fragment was subcloned into M13mp18 and 19 for sequencing purposes. 3.5 and 0.5 kb-*Bam*HI fragments from the λ ZAP *P. gingivalis* W50 DNA library were cloned into pBluescript SK(-) and M13mp18 and 19 and sequenced. Standard protocols for cDNA library screening, lambda phage purification, agarose gel electrophoresis and plasmid cloning were employed (Maniatis et al. (1982), supra). Standard protocols for cDNA library screening, lambda phage purification, agarose gel electrophoresis and plasmid cloning were employed (Maniatis et al., 1982 supra).

Example 11 Southern Blot Analysis

The membranes were washed as described above. *Bam*HI, *Hind*III- or *Pst*I-digested *P. gingivalis* H66 DNA samples were hybridized with ³²P-labeled GIN-8S-48. Two *Bam*HI fragments of approximately 9.4 and 3.5 kb, and two *Pst*I fragments of approximately 9.4 and 3 kb were found. No *Hind*III fragment was seen. *Bam*HI- and *Pst*I-digested λ DASH DNA after screening and purification of positive recombinant clones from the library revealed one clone (A1) with a 3.5 kb *Bam*HI fragment and a 3 kb *Pst*I fragment; one clone (B1) with a 9.4 kb *Bam*HI fragment and a 9.4 kb *Pst*I fragment; and 5 clones with a 9.4 kb *Bam*HI fragment and a 10 kb *Pst*I fragment. The A1 clone was sequenced because the DNA predicted to encode a 50-kDa protein is approximately 1.35 kb. In order to clone the stop codon of Arg-gingipain-2, double *Pst*I/*Hind*III-digested *P. gingivalis* DNA were hybridized with ³²P-labeled GIN-14-20. One *Pst*I/*Hind*III fragment of approximately 4.3 kb was found. This fragment was gel purified and cloned into pBluescript SK(-) for sequencing. Smaller fragments (*Pst*I/*Sma*I and *Bam*HI/*Hind*III) were also subcloned into M13mp18 and 19 and sequenced, and was found to include the stop codon. Table 2 hereinabove (see also SEQ ID NO:10) which presents about 7 kb of sequence extending from a *Pst*I site upstream of the start codon through a *Hind*III site downstream of the end of the prepolyprotein's stop codon.

Example 12 DNA Sequencing

Double-stranded DNA cloned into pBluescript SK(-) and single-stranded DNA cloned into M13mp18 and 19 were sequenced by the dideoxy terminator method [Sanger et al. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467] using sequencing kits purchased from United States Biochemicals (Cleveland, OH; Sequenase version 2.0). The DNA was sequenced using M13 universal primer, reverse sequencing primer and internal primers as well understood in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: UNIVERSITY OF GEORGIA, RESEARCH FOUNDATION INC.
- (ii) TITLE OF INVENTION: Porphyromonas Gingivalis
Arginine-Specific Proteinase Coding Sequences
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Greenlee and Winner, P.C.
 - (B) STREET: 5370 Manhattan Circle, Suite 201
 - (C) CITY: Boulder
 - (D) STATE: CO
 - (E) COUNTRY: USA
 - (F) ZIP: 80303
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: Unassigned
 - (B) FILING DATE: 09-SEP-1994
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/119,361
 - (B) FILING DATE: 10-SEP-1993
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/265,441
 - (B) FILING DATE: 24-JUN-1994
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/141,324
 - (B) FILING DATE: 21-OCT-1993
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Ferber, Donna M.
 - (B) REGISTRATION NUMBER: 33,878
 - (C) REFERENCE/DOCKET NUMBER: 21-93B PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 303-499-8080
 - (B) TELEFAX: 303-499-8089
 - (C) TELEX: 49617824

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 43 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

62

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Porphyromonas gingivalis*
(B) STRAIN: H66

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr Thr Pro Val Glu Glu Lys Gln Asn Gly Arg Met Ile Val Ile Val
1 5 10 15

Ala Lys Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp Trp Lys Asn
20 25 30

Gln Arg Gly Leu Thr Lys Xaa Val Lys Xaa Ala
35 40

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Tyr Gly Asp Ser Asn Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3159 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 949..3159

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
(B) LOCATION: 1630..3105

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGCAGAGGG CTGGTAAAGA CCGCCTCGGG ATCGAGGCCT TTGAGACGGG CACAAGCCGC	60
CGCAGCCTCC TCTTCGAAGG TGTCTCGAAC GTCCACATCG GTGAATCCGT AGCAGTGCTC	120
ATTGCCATTG AGCAGCACCG AGGTGTGGCG CATCAGATAT ATTTTCATCA GTGGATTATT	180
AGGGTATCGG TCAGAAAAAG CCTTCCGAAT CCGACAAAGA TAGTAGAAAG AGAGTGCATC	240
TGAAAACAGA TCATTCGAGG ATTATCGATC AACTGAAAAG GCAGGAGTTG TTTTGCCTTT	300
TGGTTCGGAA AATTACCTGA TCAGCATTCG TAAAAACGTG GCGCGAGAAT TTTTTCGTTT	360
TGGCGCGAGA ATTAAAAATT TTTGGAACCA CAGCGAAAAA AATCTCGCGC CGTTTTCTCA	420
GGATTTACAG ACCACAATCC GAGCATTTTC GGTTCGTAAT TCATCGAAGA GACAGGTTTT	480
ACCGCATTGA AATCAGAGAG AGAATATCCG TAGTCCAACG GTTCATCCTT ATATCAGAGG	540
TTAAAAGATA TGGTACGCTC ATCGAGGAGC TGATTGGCTT AGTAGGTGAG ACTTCTTAA	600
GAGACTATCG GCACCTACAG GAAGTTCATG GCACACAAGG CAAAGGAGGC AATCTTCGCA	660
GACCGGACTC ATATCAAAAG GATGAAACGA CTTTTCATA CGACAACCAA ATAGCCGTCT	720
ACGGTAGACG AATGCAAACC CAATATGAGG CCATCAATCA ATCCGAATGA CAGCTTTTGG	780
GCAATATATT ATGCATATTT TGATTCGCGT TTAAGGAAA AGTGCATATA TTTGCGATTG	840
TGGTATTTCT TTCGGTTTCT ATGTGAATTT TGTCTCCCAA GAAGACTTTA TAATGCATAA	900
ATACAGAAGG GGTACTACAC AGTAAAATCA TATTCTAATT TCATCAA	957
Met Lys Asn -227 -225	
TTG AAC AAG TTT GTT TCG ATT GCT CTT TGC TCT TCC TTA TTA GGA GGA	1005
Leu Asn Lys Phe Val Ser Ile Ala Leu Cys Ser Ser Leu Leu Gly Gly -220 -215 -210	
ATG GCA TTT GCG CAG CAG ACA GAG TTG GGA CGC AAT CCG AAT GTC AGA	1053
Met Ala Phe Ala Gln Thr Glu Leu Gly Arg Asn Pro Asn Val Arg -205 -200 -195	
TTG CTC GAA TCC ACT CAG CAA TCG GTG ACA AAG GTT CAG TTC CGT ATG	1101
Leu Leu Glu Ser Thr Gln Gln Ser Val Thr Lys Val Gln Phe Arg Met -190 -185 -180	
GAC AAC CTC AAG TTC ACC GAA GTT CAA ACC CCT AAG GGA ATC GGA CAA	1149
Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly Ile Gly Gln -175 -170 -165	
GTG CCG ACC TAT ACA GAA GGG GTT AAT CTT TCC GAA AAA GGG ATG CCT	1197
Val Pro Thr Tyr Thr Glu Gly Val Asn Leu Ser Glu Lys Gly Met Pro -160 -155 -150 -145	
ACG CTT CCC ATT CTA TCA CGC TCT TTG GCG GTT TCA GAC ACT CGT GAG	1245
Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp Thr Arg Glu -140 -135 -130	
ATG AAG GTA GAG GTT GTT TCC TCA AAG TTC ATC GAA AAG AAA AAT GTC	1293
Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys Lys Asn Val -125 -120 -115	
CTG ATT GCA CCC TCC AAG GGC ATG ATT ATG CGT AAC GAA GAT CCG AAA	1341
Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu Asp Pro Lys -110 -105 -100	

AAG Lys -95	ATC Ile	CCT Pro	TAC Tyr	GTT Val	TAT Tyr	GGA Gly -90	AAG Lys	AGC Ser	TAC Tyr	TCG Ser	CAA Gln -85	AAC Asn	AAA Lys	TTC Phe	TTC Phe	1389
CCG Pro -80	GGA Gly	GAG Glu	ATC Ile	GCC Ala	ACG Thr -75	CTT Leu	GAT Asp	GAT Asp	CCT Pro	TTT Phe -70	ATC Ile	CTT Leu	CGT Arg	GAT Asp	GTG Val -65	1437
CGT Arg	GGA Gly	CAG Gln	GTT Val	GTA Val -60	AAC Asn	TTT Phe	GCG Ala	CCT Pro	TTG Leu -55	CAG Gln	TAT Tyr	AAC Asn	CCT Pro	GTG Val -50	ACA Thr	1485
AAG Lys	ACG Thr	TTG Leu	CGC Arg -45	ATC Ile	TAT Tyr	ACG Thr	GAA Glu -40	ATC Ile	ACT Thr	GTG Val	GCA Ala	GTG Val	AGC Ser -35	GAA Glu	ACT Thr	1533
TCG Ser	GAA Glu	CAA Gln -30	GGC Gly	AAA Lys	AAT Asn	ATT Ile	CTG Leu -25	AAC Asn	AAG Lys	AAA Lys	GGT Gly -20	ACA Thr	TTT Phe	GCC Ala	GGC Gly	1581
TTT Phe -15	GAA Glu	GAC Asp	ACA Thr	TAC Tyr	AAG Lys	CGC Arg -10	ATG Met	TTC Phe	ATG Met	AAC Asn	TAC Tyr -5	GAG Glu	CCG Pro	GGG Gly	CGT Arg	1629
TAC Tyr 1	ACA Thr	CCG Pro	GTA Val	GAG Glu 5	GAA Glu	AAA Lys	CAA Gln	AAT Asn	GGT Gly 10	CGT Arg	ATG Met	ATC Ile	GTC Val	ATC Ile 15	GTA Val	1677
GCC Ala	AAA Lys	AAG Lys	TAT Tyr 20	GAG Glu	GGA Gly	GAT Asp	ATT Ile 25	AAA Lys	GAT Asp	TTC Phe	GTT Val	GAT Asp	TGG Trp 30	AAA Lys	AAC Asn	1725
CAA Gln	CGC Arg	GGT Gly 35	CTC Leu	CGT Arg	ACC Thr	GAG Glu 40	GTG Val 40	AAA Lys	GTG Val	GCA Ala	GAA Glu	GAT Asp 45	ATT Ile	GCT Ala	TCT Ser	1773
CCC Pro	GTT Val 50	ACA Thr	GCT Ala	AAT Asn	GCT Ala	ATT Ile 55	CAG Gln	CAG Gln	TTC Phe	GTT Val	AAG Lys 60	CAA Gln	GAA Glu	TAC Tyr	GAG Glu	1821
AAA Lys 65	GAA Glu	GGT Gly	AAT Asn	GAT Asp	TTG Leu 70	ACC Thr	TAT Tyr	GTT Val	CTT Leu	TTG Leu 75	GTT Val	GGC Gly	GAT Asp	CAC His	AAA Lys 80	1869
GAT Asp	ATT Ile	CCT Pro	GCC Ala	AAA Lys 85	ATT Ile	ACT Thr	CCG Pro	GGG Gly	ATC Ile 90	AAA Lys	TCC Ser	GAC Asp	CAG Gln	GTA Val 95	TAT Tyr	1917
GGA Gly	CAA Gln	ATA Ile	GTA Val 100	GGT Gly	AAT Asn	GAC Asp	CAC His	TAC Tyr 105	AAC Asn	GAA Glu	GTC Val	TTC Phe	ATC Ile	GGT Gly 110	CGT Arg	1965
TTC Phe	TCA Ser	TGT Cys 115	GAG Glu	AGC Ser	AAA Lys	GAG Glu	GAT Asp 120	CTG Leu	AAG Lys	ACA Thr	CAA Gln	ATC Ile	GAT Asp 125	CGG Arg	ACT Thr	2013
ATT Ile 130	CAC His	TAT Tyr	GAG Glu	CGC Arg	AAT Asn	ATA Ile 135	ACC Thr	ACG Thr	GAA Glu	GAC Asp	AAA Lys 140	TGG Trp	CTC Leu	GGT Gly	CAG Gln	2061
GCT Ala 145	CTT Leu	TGT Cys	ATT Ile	GCT Ala	TCG Ser 150	GCT Ala	GAA Glu	GGA Gly	GGC Gly	CCA Pro 155	TCC Ser	GCA Ala	GAC Asp	AAT Asn	GGT Gly 160	2109
GAA Glu	AGT Ser	GAT Asp	ATC Ile	CAG Gln 165	CAT His	GAG Glu	AAT Asn	GTA Val	ATC Ile 170	GCC Ala	AAT Asn	CTG Leu	CTT Leu	ACC Thr	CAG Gln 175	2157

TAT GGC TAT ACC AAG ATT ATC AAA TGT TAT GAT CCG GGA GTA ACT CCT Tyr Gly Tyr Thr Lys Ile Ile Lys Cys Tyr Asp Pro Gly Val Thr Pro 180 185 190	2205
AAA AAC ATT ATT GAT GCT TTC AAC GGA GGA ATC TCG TTG GTC AAC TAT Lys Asn Ile Ile Asp Ala Phe Asn Gly Gly Ile Ser Leu Val Asn Tyr 195 200 205	2253
ACG GGC CAC GGT AGC GAA ACA GCT TGG GGT ACG TCT CAC TTC GGC ACC Thr Gly His Gly Ser Glu Thr Ala Trp Gly Thr Ser His Phe Gly Thr 210 215 220	2301
ACT CAT GTG AAG CAG CTT ACC AAC AGC AAC CAG CTA CCG TTT ATT TTC Thr His Val Lys Gln Leu Thr Asn Ser Asn Gln Leu Pro Phe Ile Phe 225 230 235 240	2349
GAC GTA GCT TGT GTG AAT GGC GAT TTC CTA TTC AGC ATG CCT TGC TTC Asp Val Ala Cys Val Asn Gly Asp Phe Leu Phe Ser Met Pro Cys Phe 245 250 255	2397
GCA GAA GCC CTG ATG CGT GCA CAA AAA GAT GGT AAG CCG ACA GGT ACT Ala Glu Ala Leu Met Arg Ala Gln Lys Asp Gly Lys Pro Thr Gly Thr 260 265 270	2445
GTT GCT ATC ATA GCG TCT ACG ATC AAC CAG TCT TGG GCT TCT CCT ATG Val Ala Ile Ile Ala Ser Thr Ile Asn Gln Ser Trp Ala Ser Pro Met 275 280 285	2493
CGC GGG CAG GAT GAG ATG AAC GAA ATT CTG TGC GAA AAA CAC CCG AAC Arg Gly Gln Asp Glu Met Asn Glu Ile Leu Cys Glu Lys His Pro Asn 290 295 300	2541
AAC ATC AAG CGT ACT TTC GGT GGT GTC ACC ATG AAC GGT ATG TTT GCT Asn Ile Lys Arg Thr Phe Gly Gly Val Thr Met Asn Gly Met Phe Ala 305 310 315 320	2589
ATG GTG GAA AAG TAT AAA AAG GAT GGT GAG AAG ATG CTC GAC ACA TGG Met Val Glu Lys Tyr Lys Lys Asp Gly Glu Lys Met Leu Asp Thr Trp 325 330 335	2637
ACT GTT TTC GGC GAC CCC TCG CTG CTC GTT CGT ACA CTT GTC CCG ACC Thr Val Phe Gly Asp Pro Ser Leu Leu Val Arg Thr Leu Val Pro Thr 340 345 350	2685
AAA ATG CAG GTT ACG GCT CCG GCT CAG ATT AAT TTG ACG GAT GCT TCA Lys Met Gln Val Thr Ala Pro Ala Gln Ile Asn Leu Thr Asp Ala Ser 355 360 365	2733
GTC AAC GTA TCT TGC GAT TAT AAT GGT GCT ATT GCT ACC ATT TCA GCC Val Asn Val Ser Cys Asp Tyr Asn Gly Ala Ile Ala Thr Ile Ser Ala 370 375 380	2781
AAT GGA AAG ATG TTC GGT TCT GCA GTT GTC GAA AAT GGA ACA GCT ACA Asn Gly Lys Met Phe Gly Ser Ala Val Val Glu Asn Gly Thr Ala Thr 385 390 395 400	2829
ATC AAT CTG ACA GGT CTG ACA AAT GAA AGC ACG CTT ACC CTT ACA GTA Ile Asn Leu Thr Gly Leu Thr Asn Glu Ser Thr Leu Thr Leu Thr Val 405 410 415	2877
GTT GGT TAC AAC AAA GAG ACG GTT ATT AAG ACC ATC AAC ACT AAT GGT Val Gly Tyr Asn Lys Glu Thr Val Ile Lys Thr Ile Asn Thr Asn Gly 420 425 430	2925
GAG CCT AAC CCC TAC CAG CCC GTT TCC AAC TTG ACA GCT ACA ACG CAG Glu Pro Asn Pro Tyr Gln Pro Val Ser Asn Leu Thr Ala Thr Thr Gln 435 440 445	2973

66

GGT CAG AAA GTA ACG CTC AAG TGG GAT GCA CCG AGC ACG AAA ACC AAT	3021
Gly Gln Lys Val Thr Leu Lys Trp Asp Ala Pro Ser Thr Lys Thr Asn	
450 455 460	
GCA ACC ACT AAT ACC GCT CGC AGC GTG GAT GGC ATA CGA GAA TTG GTT	3069
Ala Thr Thr Asn Thr Ala Arg Ser Val Asp Gly Ile Arg Glu Leu Val	
465 470 475 480	
CTT CTG TCA GTC AGC GAT GCC CCC GAA CTT CTT CGC AGC GGT CAG GCC	3117
Leu Leu Ser Val Ser Asp Ala Pro Glu Leu Arg Ser Gly Gln Ala	
485 490 495	
GAG ATT GTT CTT GAA GCT CAC GAT GTT TGG AAT GAT GGA TCC	3159
Glu Ile Val Leu Glu Ala His Asp Val Trp Asn Asp Gly Ser	
500 505 510	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 737 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Lys Asn Leu Asn Lys Phe Val Ser Ile Ala Leu Cys Ser Ser Leu	
-227 -225 -220 -215	
Leu Gly Gly Met Ala Phe Ala Gln Gln Thr Glu Leu Gly Arg Asn Pro	
-210 -205 -200	
Asn Val Arg Leu Leu Glu Ser Thr Gln Gln Ser Val Thr Lys Val Gln	
-195 -190 -185 -180	
Phe Arg Met Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly	
-175 -170 -165	
Ile Gly Gln Val Pro Thr Tyr Thr Glu Gly Val Asn Leu Ser Glu Lys	
-160 -155 -150	
Gly Met Pro Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp	
-145 -140 -135	
Thr Arg Glu Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys	
-130 -125 -120	
Lys Asn Val Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu	
-115 -110 -105 -100	
Asp Pro Lys Lys Ile Pro Tyr Val Tyr Gly Lys Ser Tyr Ser Gln Asn	
-95 -90 -85	
Lys Phe Phe Pro Gly Glu Ile Ala Thr Leu Asp Asp Pro Phe Ile Leu	
-80 -75 -70	
Arg Asp Val Arg Gly Gln Val Val Asn Phe Ala Pro Leu Gln Tyr Asn	
-65 -60 -55	
Pro Val Thr Lys Thr Leu Arg Ile Tyr Thr Glu Ile Thr Val Ala Val	
-50 -45 -40	
Ser Glu Thr Ser Glu Gln Gly Lys Asn Ile Leu Asn Lys Lys Gly Thr	
-35 -30 -25 -20	

Phe	Ala	Gly	Phe	Glu -15	Asp	Thr	Tyr	Lys	Arg -10	Met	Phe	Met	Asn	Tyr -5	Glu
Pro	Gly	Arg	Tyr 1	Thr	Pro	Val	Glu 5	Glu	Lys	Gln	Asn	Gly 10	Arg	Met	Ile
Val	Ile 15	Val	Ala	Lys	Lys	Tyr 20	Glu	Gly	Asp	Ile	Lys 25	Asp	Phe	Val	Asp
Trp	Lys	Asn	Gln	Arg	Gly 35	Leu	Arg	Thr	Glu	Val 40	Lys	Val	Ala	Glu	Asp 45
Ile	Ala	Ser	Pro	Val 50	Thr	Ala	Asn	Ala	Ile 55	Gln	Gln	Phe	Val	Lys 60	Gln
Glu	Tyr	Glu	Lys 65	Glu	Gly	Asn	Asp	Leu 70	Thr	Tyr	Val	Leu	Leu 75	Val	Gly
Asp	His	Lys 80	Asp	Ile	Pro	Ala	Lys 85	Ile	Thr	Pro	Gly	Ile 90	Lys	Ser	Asp
Gln	Val 95	Tyr	Gly	Gln	Ile	Val 100	Gly	Asn	Asp	His	Tyr 105	Asn	Glu	Val	Phe
Ile 110	Gly	Arg	Phe	Ser	Cys 115	Glu	Ser	Lys	Glu	Asp 120	Leu	Lys	Thr	Gln	Ile 125
Asp	Arg	Thr	Ile	His 130	Tyr	Glu	Arg	Asn	Ile 135	Thr	Thr	Glu	Asp	Lys 140	Trp
Leu	Gly	Gln	Ala 145	Leu	Cys	Ile	Ala	Ser 150	Ala	Glu	Gly	Gly	Pro 155	Ser	Ala
Asp	Asn	Gly 160	Glu	Ser	Asp	Ile	Gln 165	His	Glu	Asn	Val	Ile 170	Ala	Asn	Leu
Leu	Thr 175	Gln	Tyr	Gly	Tyr	Thr 180	Lys	Ile	Ile	Lys	Cys 185	Tyr	Asp	Pro	Gly
Val 190	Thr	Pro	Lys	Asn	Ile 195	Ile	Asp	Ala	Phe	Asn 200	Gly	Gly	Ile	Ser	Leu 205
Val	Asn	Tyr	Thr	Gly 210	His	Gly	Ser	Glu	Thr 215	Ala	Trp	Gly	Thr	Ser 220	His
Phe	Gly	Thr	Thr 225	His	Val	Lys	Gln	Leu 230	Thr	Asn	Ser	Asn	Gln 235	Leu	Pro
Phe	Ile	Phe 240	Asp	Val	Ala	Cys	Val 245	Asn	Gly	Asp	Phe	Leu 250	Phe	Ser	Met
Pro	Cys 255	Phe	Ala	Glu	Ala	Leu 260	Met	Arg	Ala	Gln	Lys 265	Asp	Gly	Lys	Pro
Thr 270	Gly	Thr	Val	Ala	Ile 275	Ile	Ala	Ser	Thr	Ile 280	Asn	Gln	Ser	Trp	Ala 285
Ser	Pro	Met	Arg	Gly 290	Gln	Asp	Glu	Met	Asn 295	Glu	Ile	Leu	Cys	Glu 300	Lys
His	Pro	Asn	Asn 305	Ile	Lys	Arg	Thr	Phe 310	Gly	Gly	Val	Thr	Met 315	Asn	Gly
Met	Phe 320	Ala	Met	Val	Glu	Lys	Tyr 325	Lys	Lys	Asp	Gly	Glu 330	Lys	Met	Leu

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Asp Thr Trp Thr Val Phe Gly Asp Pro Ser Leu Leu Val Arg Thr Leu
 335 340 345
 Val Pro Thr Lys Met Gln Val Thr Ala Pro Ala Gln Ile Asn Leu Thr
 350 355 360 365
 Asp Ala Ser Val Asn Val Ser Cys Asp Tyr Asn Gly Ala Ile Ala Thr
 370 375 380
 Ile Ser Ala Asn Gly Lys Met Phe Gly Ser Ala Val Val Glu Asn Gly
 385 390 395
 Thr Ala Thr Ile Asn Leu Thr Gly Leu Thr Asn Glu Ser Thr Leu Thr
 400 405 410
 Leu Thr Val Val Gly Tyr Asn Lys Glu Thr Val Ile Lys Thr Ile Asn
 415 420 425
 Thr Asn Gly Glu Pro Asn Pro Tyr Gln Pro Val Ser Asn Leu Thr Ala
 430 435 440 445
 Thr Thr Gln Gly Gln Lys Val Thr Leu Lys Trp Asp Ala Pro Ser Thr
 450 455 460
 Lys Thr Asn Ala Thr Thr Asn Thr Ala Arg Ser Val Asp Gly Ile Arg
 465 470 475
 Glu Leu Val Leu Leu Ser Val Ser Asp Ala Pro Glu Leu Leu Arg Ser
 480 485 490
 Gly Gln Ala Glu Ile Val Leu Glu Ala His Asp Val Trp Asn Asp Gly
 495 500 505
 Ser
 510

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Glu Leu Leu Arg
1

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (other nucleic acid)
- (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGCTTTACNC CNGTNGARGA RYTNGA

26

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (other nucleic acid)
- (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCTTTRTTY TTCCARTCNA CRAARTCYTT

30

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 48 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (other nucleic acid)
- (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTGGAGAAT TCTCGTATGA TCGTCATCGT AGCCAAAAAG TATGAGGG

48

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (other nucleic acid)
- (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATCAACACTA ATGGTGAGCC

20

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:

70

(A) LENGTH: 7266 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 949..6063

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTGCAGAGGG CTGGTAAAGA CCGCCTCGGG ATCGAGGCCT TTGAGACGGG CACAAGCCGC	60
CGCAGCCTCC TCTTCGAAGG TGTCTCGAAC GTCCACATCG GTGAATCCGT AGCAGTGCTC	120
ATTGCCATTG AGCAGCACCG AGGTGTGGCG CATCAGATAT ATTTTCATCA GTGGATTATT	180
AGGGTATCGG TCAGAAAAAG CCTTCCGAAT CCGACAAAGA TAGTAGAAAG AGAGTGCATC	240
TGAAAACAGA TCATTGAGG ATTATCGATC AACTGAAAAG GCAGGAGTTG TTTTGC GTTT	300
TGGTTCGGAA AATTACCTGA TCAGCATTG TAAAAACGTG GCGCGAGAAT TTTTTC GTTT	360
TGGCGCGAGA ATTAAAAATT TTTGGAACCA CAGCGAAAAA AATCTCGCGC CGTTTTCTCA	420
GGATTACAG ACCACAATCC GAGCATTTC GGTTCGTAAT TCATCGAAGA GACAGGTTTT	480
ACCGCATTGA AATCAGAGAG AGAATATCCG TAGTCCAACG GTTCATCCTT ATATCAGAGG	540
TTAAAAGATA TGGTACGCTC ATCGAGGAGC TGATTGGCTT AGTAGGTGAG ACTTTCTTAA	600
GAGACTATCG GCACCTACAG GAAGTTCATG GCACACAAGG CAAAGGAGGC AATCTTCGCA	660
GACCGGACTC ATATCAAAAG GATGAAACGA CTTTTCCATA CGACAACCAA ATAGCCGTCT	720
ACGGTAGACG AATGCAAACC CAATATGAGG CCATCAATCA ATCCGAATGA CAGCTTTTGG	780
GCAATATATT ATGCATATTT TGATTCGCGT TTAAAGGAAA AGTGCATATA TTTGCGATTG	840
TGGTATTTCT TTCGGTTTCT ATGTGAATTT TGTCTCCCAA GAAGACTTTA TAATGCATAA	900
ATACAGAAGG GGTACTACAC AGTAAAATCA TATTCTAATT TCATCAAA ATG AAA AAC	957
	Met Lys Asn
	1
TTG AAC AAG TTT GTT TCG ATT GCT CTT TGC TCT TCC TTA TTA GGA GGA	1005
Leu Asn Lys Phe Val Ser Ile Ala Leu Cys Ser Ser Leu Leu Gly Gly	
5 10 15	
ATG GCA TTT GCG CAG CAG ACA GAG TTG GGA CGC AAT CCG AAT GTC AGA	1053
Met Ala Phe Ala Gln Gln Thr Glu Leu Gly Arg Asn Pro Asn Val Arg	
20 25 30 35	
TTG CTC GAA TCC ACT CAG CAA TCG GTG ACA AAG GTT CAG TTC CGT ATG	1101
Leu Leu Glu Ser Thr Gln Gln Ser Val Thr Lys Val Gln Phe Arg Met	
40 45 50	

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GAC AAC CTC AAG TTC ACC GAA GTT CAA ACC CCT AAG GGA ATC GGA CAA Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly Ile Gly Gln 55 60 65	1149
GTG CCG ACC TAT ACA GAA GGG GTT AAT CTT TCC GAA AAA GGG ATG CCT Val Pro Thr Tyr Thr Glu Gly Val Asn Leu Ser Glu Lys Gly Met Pro 70 75 80	1197
ACG CTT CCC ATT CTA TCA CGC TCT TTG GCG GTT TCA GAC ACT CGT GAG Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp Thr Arg Glu 85 90 95	1245
ATG AAG GTA GAG GTT GTT TCC TCA AAG TTC ATC GAA AAG AAA AAT GTC Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys Lys Asn Val 100 105 110 115	1293
CTG ATT GCA CCC TCC AAG GGC ATG ATT ATG CGT AAC GAA GAT CCG AAA Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu Asp Pro Lys 120 125 130	1341
AAG ATC CCT TAC GTT TAT GGA AAG AGC TAC TCG CAA AAC AAA TTC TTC Lys Ile Pro Tyr Val Tyr Gly Lys Ser Tyr Ser Gln Asn Lys Phe Phe 135 140 145	1389
CCG GGA GAG ATC GCC ACG CTT GAT GAT CCT TTT ATC CTT CGT GAT GTG Pro Gly Glu Ile Ala Thr Leu Asp Asp Pro Phe Ile Leu Arg Asp Val 150 155 160	1437
CGT GGA CAG GTT GTA AAC TTT GCG CCT TTG CAG TAT AAC CCT GTG ACA Arg Gly Gln Val Val Asn Phe Ala Pro Leu Gln Tyr Asn Pro Val Thr 165 170 175	1485
AAG ACG TTG CGC ATC TAT ACG GAA ATC ACT GTG GCA GTG AGC GAA ACT Lys Thr Leu Arg Ile Tyr Thr Glu Ile Thr Val Ala Val Ser Glu Thr 180 185 190 195	1533
TCG GAA CAA GGC AAA AAT ATT CTG AAC AAG AAA GGT ACA TTT GCC GGC Ser Glu Gln Gly Lys Asn Ile Leu Asn Lys Lys Gly Thr Phe Ala Gly 200 205 210	1581
TTT GAA GAC ACA TAC AAG CGC ATG TTC ATG AAC TAC GAG CCG GGG CGT Phe Glu Asp Thr Tyr Lys Arg Met Phe Met Asn Tyr Glu Pro Gly Arg 215 220 225	1629
TAC ACA CCG GTA GAG GAA AAA CAA AAT GGT CGT ATG ATC GTC ATC GTA Tyr Thr Pro Val Glu Glu Lys Gln Asn Gly Arg Met Ile Val Ile Val 230 235 240	1677
GCC AAA AAG TAT GAG GGA GAT ATT AAA GAT TTC GTT GAT TGG AAA AAC Ala Lys Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp Trp Lys Asn 245 250 255	1725
CAA CGC GGT CTC CGT ACC GAG GTG AAA GTG GCA GAA GAT ATT GCT TCT Gln Arg Gly Leu Arg Thr Glu Val Lys Val Ala Glu Asp Ile Ala Ser 260 265 270 275	1773
CCC GTT ACA GCT AAT GCT ATT CAG CAG TTC GTT AAG CAA GAA TAC GAG Pro Val Thr Ala Asn Ala Ile Gln Gln Phe Val Lys Gln Glu Tyr Glu 280 285 290	1821
AAA GAA GGT AAT GAT TTG ACC TAT GTT CTT TTG GTT GGC GAT CAC AAA Lys Glu Gly Asn Asp Leu Thr Tyr Val Leu Leu Val Gly Asp His Lys 295 300 305	1869

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GAT Asp	ATT Ile	CCT Pro	GCC Ala	AAA Lys	ATT Ile	ACT Thr	CCG Pro	GGG Gly	ATC Ile	AAA Lys	TCC Ser	GAC Asp	CAG Gln	GTA Val	TAT Tyr	1917
	310						315					320				
GGA Gly	CAA Gln	ATA Ile	GTA Val	GGT Gly	AAT Asn	GAC Asp	CAC His	TAC Tyr	AAC Asn	GAA Glu	GTC Val	TTC Phe	ATC Ile	GGT Gly	CGT Arg	1965
	325					330					335					
TTC Phe	TCA Ser	TGT Cys	GAG Glu	AGC Ser	AAA Lys	GAG Glu	GAT Asp	CTG Leu	AAG Lys	ACA Thr	CAA Gln	ATC Ile	GAT Asp	CGG Arg	ACT Thr	2013
	340				345					350					355	
ATT Ile	CAC His	TAT Tyr	GAG Glu	CGC Arg	AAT Asn	ATA Ile	ACC Thr	ACG Thr	GAA Glu	GAC Asp	AAA Lys	TGG Trp	CTC Leu	GGT Gly	CAG Gln	2061
				360					365					370		
GCT Ala	CTT Leu	TGT Cys	ATT Ile	GCT Ala	TCG Ser	GCT Ala	GAA Glu	GGA Gly	GGC Gly	CCA Pro	TCC Ser	GCA Ala	GAC Asp	AAT Asn	GGT Gly	2109
			375					380					385			
GAA Glu	AGT Ser	GAT Asp	ATC Ile	CAG Gln	CAT His	GAG Glu	AAT Asn	GTA Val	ATC Ile	GCC Ala	AAT Asn	CTG Leu	CTT Leu	ACC Thr	CAG Gln	2157
	390						395					400				
TAT Tyr	GGC Gly	TAT Tyr	ACC Thr	AAG Lys	ATT Ile	ATC Ile	AAA Lys	TGT Cys	TAT Tyr	GAT Asp	CCG Pro	GGA Gly	GTA Val	ACT Thr	CCT Pro	2205
	405					410					415					
AAA Lys	AAC Asn	ATT Ile	ATT Ile	GAT Asp	GCT Ala	TTC Phe	AAC Asn	GGA Gly	GGA Gly	ATC Ile	TCG Ser	TTG Leu	GTC Val	AAC Asn	TAT Tyr	2253
	420				425					430					435	
ACG Thr	GGC Gly	CAC His	GGT Gly	AGC Ser	GAA Glu	ACA Thr	GCT Ala	TGG Trp	GGT Gly	ACG Thr	TCT Ser	CAC His	TTC Phe	GGC Gly	ACC Thr	2301
				440					445					450		
ACT Thr	CAT His	GTG Val	AAG Lys	CAG Gln	CTT Leu	ACC Thr	AAC Asn	AGC Ser	AAC Asn	CAG Gln	CTA Leu	CCG Pro	TTT Phe	ATT Ile	TTC Phe	2349
			455					460					465			
GAC Asp	GTA Val	GCT Ala	TGT Cys	GTG Val	AAT Asn	GGC Gly	GAT Asp	TTC Phe	CTA Leu	TTC Phe	AGC Ser	ATG Met	CCT Pro	TGC Cys	TTC Phe	2397
		470					475					480				
GCA Ala	GAA Glu	GCC Ala	CTG Leu	ATG Met	CGT Arg	GCA Ala	CAA Gln	AAA Lys	GAT Asp	GGT Gly	AAG Lys	CCG Pro	ACA Thr	GGT Gly	ACT Thr	2445
	485					490					495					
GTT Val	GCT Ala	ATC Ile	ATA Ile	GCG Ala	TCT Ser	ACG Thr	ATC Ile	AAC Asn	CAG Gln	TCT Ser	TGG Trp	GCT Ala	TCT Ser	CCT Pro	ATG Met	2493
	500				505					510					515	
CGC Arg	GGG Gly	CAG Gln	GAT Asp	GAG Glu	ATG Met	AAC Asn	GAA Glu	ATT Ile	CTG Leu	TGC Cys	GAA Glu	AAA Lys	CAC His	CCG Pro	AAC Asn	2541
				520					525					530		
AAC Asn	ATC Ile	AAG Lys	CGT Arg	ACT Thr	TTC Phe	GGT Gly	GGT Gly	GTC Val	ACC Thr	ATG Met	AAC Asn	GGT Gly	ATG Met	TTT Phe	GCT Ala	2589
			535					540					545			
ATG Met	GTG Val	GAA Glu	AAG Lys	TAT Tyr	AAA Lys	AAG Lys	GAT Asp	GGT Gly	GAG Glu	AAG Lys	ATG Met	CTC Leu	GAC Asp	ACA Thr	TGG Trp	2637
		550					555					560				

ACT Thr 565	GTT Val	TTC Phe	GGC Gly	GAC Asp	CCC Pro	TCG Ser	CTG Leu	CTC Leu	GTT Val	CGT Arg	ACA Thr	CTT Leu	GTC Val	CCG Pro	ACC Thr	2685
AAA Lys 580	ATG Met	CAG Gln	GTT Val	ACG Thr	GCT Ala	CCG Pro	GCT Ala	CAG Gln	ATT Ile	AAT Asn	TTG Leu	ACG Thr	GAT Asp	GCT Ala	TCA Ser	2733
GTC Val	AAC Asn	GTA Val	TCT Ser	TGC Cys	GAT Asp	TAT Tyr	AAT Asn	GGT Gly	GCT Ala	ATT Ile	GCT Ala	ACC Thr	ATT Ile	TCA Ser	GCC Ala	2781
AAT Asn	GGA Gly	AAG Lys	ATG Met	TTC Phe	GGT Gly	TCT Ser	GCA Ala	GTT Val	GTC Val	GAA Glu	AAT Asn	GGA Gly	ACA Thr	GCT Ala	ACA Thr	2829
ATC Ile	AAT Asn	CTG Leu	ACA Thr	GGT Gly	CTG Leu	ACA Thr	AAT Asn	GAA Glu	AGC Ser	ACG Thr	CTT Leu	ACC Thr	CTT Leu	ACA Thr	GTA Val	2877
GTT Val	GGT Gly	TAC Tyr	AAC Asn	AAA Lys	GAG Glu	ACG Thr	GTT Val	ATT Ile	AAG Lys	ACC Thr	ATC Ile	AAC Asn	ACT Thr	AAT Asn	GGT Gly	2925
GAG Glu	CCT Pro	AAC Asn	CCC Pro	TAC Tyr	CAG Gln	CCC Pro	GTT Val	TCC Ser	AAC Asn	TTG Leu	ACA Thr	GCT Ala	ACA Thr	ACG Thr	CAG Gln	2973
GGT Gly	CAG Gln	AAA Lys	GTA Val	ACG Thr	CTC Leu	AAG Lys	TGG Trp	GAT Asp	GCA Ala	CCG Pro	AGC Ser	ACG Thr	AAA Lys	ACC Thr	AAT Asn	3021
GCA Ala	ACC Thr	ACT Thr	AAT Asn	ACC Thr	GCT Ala	CGC Arg	AGC Ser	GTG Val	GAT Asp	GGC Gly	ATA Ile	CGA Arg	GAA Glu	TTG Leu	GTT Val	3069
CTT Leu	CTG Leu	TCA Ser	GTC Val	AGC Ser	GAT Asp	GCC Ala	CCC Pro	GAA Glu	CTT Leu	CTT Leu	CGC Arg	AGC Ser	GGT Gly	CAG Gln	GCC Ala	3117
GAG Glu	ATT Ile	GTT Val	CTT Leu	GAA Glu	GCT Ala	CAC His	GAT Asp	GTT Val	TGG Trp	AAT Asn	GAT Asp	GGA Gly	TCC Ser	GGT Gly	TAT Tyr	3165
CAG Gln	ATT Ile	CTT Leu	TTG Leu	GAT Asp	GCA Ala	GAC Asp	CAT His	GAT Asp	CAA Gln	TAT Tyr	GGA Gly	CAG Gln	GTT Val	ATA Ile	CCC Pro	3213
AGT Ser	GAT Asp	ACC Thr	CAT His	ACT Thr	CTT Leu	TGG Trp	CCG Pro	AAC Asn	TGT Cys	AGT Ser	GTC Val	CCG Pro	GCC Ala	AAT Asn	CTG Leu	3261
TTC Phe	GCT Ala	CCG Pro	TTC Phe	GAA Glu	TAT Tyr	ACT Thr	GTT Val	CCG Pro	GAA Glu	AAT Asn	GCA Ala	GAT Asp	CCT Pro	TCT Ser	TGT Cys	3309
TCC Ser	CCT Pro	ACC Thr	AAT Asn	ATG Met	ATA Ile	ATG Met	GAT Asp	GGT Gly	ACT Thr	GCA Ala	TCC Ser	GTT Val	AAT Asn	ATA Ile	CCG Pro	3357
GCC Ala	GGA Gly	ACT Thr	TAT Tyr	GAC Asp	TTT Phe	GCA Ala	ATT Ile	GCT Ala	GCT Ala	CCT Pro	CAA Gln	GCA Ala	AAT Asn	GCA Ala	AAG Lys	3405
ATT Ile	TGG Trp	ATT Ile	GCC Ala	GGA Gly	CAA Gln	GGA Gly	CCG Pro	ACG Thr	AAA Lys	GAA Glu	GAT Asp	GAT Asp	TAT Tyr	GTA Val	TTT Phe	3453

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GAA GCC GGT AAA AAA TAC CAT TTC CTT ATG AAG AAG ATG GGT AGC GGT Glu Ala Gly Lys Lys Tyr His Phe Leu Met Lys Lys Met Gly Ser Gly	3501
840 845 850	
GAT GGA ACT GAA TTG ACT ATA AGC GAA GGT GGT GGA AGC GAT TAC ACC Asp Gly Thr Glu Leu Thr Ile Ser Glu Gly Gly Gly Ser Asp Tyr Thr	3549
855 860 865	
TAT ACT GTC TAT CGT GAC GGC ACG AAG ATC AAG GAA GGT CTG ACG GCT Tyr Thr Val Tyr Arg Asp Gly Thr Lys Ile Lys Glu Gly Leu Thr Ala	3597
870 875 880	
ACG ACA TTC GAA GAA GAC GGT GTA GCT ACG GGC AAT CAT GAG TAT TGC Thr Thr Phe Glu Glu Asp Gly Val Ala Thr Gly Asn His Glu Tyr Cys	3645
885 890 895	
GTG GAA GTT AAG TAC ACA GCC GGC GTA TCT CCG AAG GTA TGT AAA GAC Val Glu Val Lys Tyr Thr Ala Gly Val Ser Pro Lys Val Cys Lys Asp	3693
900 905 910 915	
GTT ACG GTA GAA GGA TCC AAT GAA TTT GCT CCT GTA CAG AAC CTG ACC Val Thr Val Glu Gly Ser Asn Glu Phe Ala Pro Val Gln Asn Leu Thr	3741
920 925 930	
GGT AGT GCA GTC GGC CAG AAA GTA ACG CTC AAG TGG GAT GCA CCT AAT Gly Ser Ala Val Gly Gln Lys Val Thr Leu Lys Trp Asp Ala Pro Asn	3789
935 940 945	
GGT ACC CCG AAT CCA AAT CCG AAT CCG AAT CCG AAT CCC GGA ACA ACA Gly Thr Pro Asn Pro Asn Pro Asn Pro Asn Pro Asn Pro Gly Thr Thr	3837
950 955 960	
ACA CTT TCC GAA TCA TTC GAA AAT GGT ATT CCT GCC TCA TGG AAG ACG Thr Leu Ser Glu Ser Phe Glu Asn Gly Ile Pro Ala Ser Trp Lys Thr	3885
965 970 975	
ATC GAT GCA GAC GGT GAC GGG CAT GGC TGG AAG CCT GGA AAT GCT CCC Ile Asp Ala Asp Gly Asp Gly His Gly Trp Lys Pro Gly Asn Ala Pro	3933
980 985 990 995	
GGA ATC GCT GGC TAC AAT AGC AAT GGT TGT GTA TAT TCA GAG TCA TTC Gly Ile Ala Gly Tyr Asn Ser Asn Gly Cys Val Tyr Ser Glu Ser Phe	3981
1000 1005 1010	
GGT CTT GGT GGT ATA GGA GTT CTT ACC CCT GAC AAC TAT CTG ATA ACA Gly Leu Gly Gly Ile Gly Val Leu Thr Pro Asp Asn Tyr Leu Ile Thr	4029
1015 1020 1025	
CCG GCA TTG GAT TTG CCT AAC GGA GGT AAG TTG ACT TTC TGG GTA TGC Pro Ala Leu Asp Leu Pro Asn Gly Gly Lys Leu Thr Phe Trp Val Cys	4077
1030 1035 1040	
GCA CAG GAT GCT AAT TAT GCA TCC GAG CAC TAT GCG GTG TAT GCA TCT Ala Gln Asp Ala Asn Tyr Ala Ser Glu His Tyr Ala Val Tyr Ala Ser	4125
1045 1050 1055	
TCG ACC GGT AAC GAT GCA TCC AAC TTC ACG AAT GCT TTG TTG GAA GAG Ser Thr Gly Asn Asp Ala Ser Asn Phe Thr Asn Ala Leu Leu Glu Glu	4173
1060 1065 1070 1075	
ACG ATT ACG GCA AAA GGT GTT CGC TCG CCG GAA GCT ATT CGT GGT CGT Thr Ile Thr Ala Lys Gly Val Arg Ser Pro Glu Ala Ile Arg Gly Arg	4221
1080 1085 1090	
ATA CAG GGT ACT TGG CGC CAG AAG ACG GTA GAC CTT CCC GCA GGT ACG Ile Gln Gly Thr Trp Arg Gln Lys Thr Val Asp Leu Pro Ala Gly Thr	4269
1095 1100 1105	

AAA TAT GTT GCT TTC CGT CAC TTC CAA AGC ACG GAT ATG TTC TAC ATC Lys Tyr Val Ala Phe Arg His Phe Gln Ser Thr Asp Met Phe Tyr Ile 1110 1115 1120	4317
GAC CTT GAT GAG GTT GAG ATC AAG GCC AAC GGC AAG CGC GCA GAC TTC Asp Leu Asp Glu Val Glu Ile Lys Ala Asn Gly Lys Arg Ala Asp Phe 1125 1130 1135	4365
ACG GAA ACG TTC GAG TCT TCT ACT CAT GGA GAG GCA CCG GCG GAA TGG Thr Glu Thr Phe Glu Ser Ser Thr His Gly Glu Ala Pro Ala Glu Trp 1140 1145 1150 1155	4413
ACT ACT ATC GAT GCC GAT GGC GAT GGT CAG GGT TGG CTC TGT CTG TCT Thr Thr Ile Asp Ala Asp Gly Asp Gly Gln Gly Trp Leu Cys Leu Ser 1160 1165 1170	4461
TCC GGA CAA TTG GAC TGG CTG ACA GCT CAT GGC GGC ACC AAC GTA GTA Ser Gly Gln Leu Asp Trp Leu Thr Ala His Gly Gly Thr Asn Val Val 1175 1180 1185	4509
GCC TCT TTC TCA TGG AAT GGA ATG GCT TTG AAT CCT GAT AAC TAT CTC Ala Ser Phe Ser Trp Asn Gly Met Ala Leu Asn Pro Asp Asn Tyr Leu 1190 1195 1200	4557
ATC TCA AAG GAT GTT ACA GGC GCA ACG AAG GTA AAG TAC TAC TAT GCA Ile Ser Lys Asp Val Thr Gly Ala Thr Lys Val Lys Tyr Tyr Tyr Ala 1205 1210 1215	4605
GTC AAC GAC GGT TTT CCC GGG GAT CAC TAT GCG GTG ATG ATC TCC AAG Val Asn Asp Gly Phe Pro Gly Asp His Tyr Ala Val Met Ile Ser Lys 1220 1225 1230 1235	4653
ACG GGC ACG AAC GCC GGA GAC TTC ACG GTT GTT TTC GAA GAA ACG CCT Thr Gly Thr Asn Ala Gly Asp Phe Thr Val Val Phe Glu Glu Thr Pro 1240 1245 1250	4701
AAC GGA ATA AAT AAG GGC GGA GCA AGA TTC GGT CTT TCC ACG GAA GCC Asn Gly Ile Asn Lys Gly Gly Ala Arg Phe Gly Leu Ser Thr Glu Ala 1255 1260 1265	4749
AAT GGC GCC AAA CCT CAA AGT GTA TGG ATC GAG CGT ACG GTA GAT TTG Asn Gly Ala Lys Pro Gln Ser Val Trp Ile Glu Arg Thr Val Asp Leu 1270 1275 1280	4797
CCT GCG GGC ACG AAG TAT GTT GCT TTC CGT CAC TAC AAT TGC TCG GAT Pro Ala Gly Thr Lys Tyr Val Ala Phe Arg His Tyr Asn Cys Ser Asp 1285 1290 1295	4845
TTG AAC TAC ATT CTT TTG GAT GAT ATT CAG TTC ACC ATG GGT GGC AGC Leu Asn Tyr Ile Leu Leu Asp Asp Ile Gln Phe Thr Met Gly Gly Ser 1300 1305 1310 1315	4893
CCC ACC CCG ACC GAT TAT ACC TAC ACG GTG TAT CGT GAC GGT ACG AAG Pro Thr Pro Thr Asp Tyr Thr Tyr Thr Val Tyr Arg Asp Gly Thr Lys 1320 1325 1330	4941
ATC AAG GAA GGT CTG ACC GAA ACG ACC TTC GAA GAA GAC GGC GTA GCT Ile Lys Glu Gly Leu Thr Glu Thr Thr Phe Glu Glu Asp Gly Val Ala 1335 1340 1345	4989
ACA GGC AAT CAT GAG TAT TGC GTG GAA GTG AAG TAC ACA GCC GGC GTA Thr Gly Asn His Glu Tyr Cys Val Glu Val Lys Tyr Thr Ala Gly Val 1350 1355 1360	5037
TCT CCG AAA GAG TGC GTA AAC GTA ACT ATT AAT CCG ACT CAG TTC AAT Ser Pro Lys Glu Cys Val Asn Val Thr Ile Asn Pro Thr Gln Phe Asn 1365 1370 1375	5085

CCT GTA AAG AAC CTG AAG GCA CAA CCG GAT GGC GGC GAC GTG GTT CTC Pro Val Lys Asn Leu Lys Ala Gln Pro Asp Gly Gly Asp Val Val Leu 1380 1385 1390 1395	5133
AAG TGG GAA GCC CCG AGC GCA AAA AAG ACA GAA GGT TCT CGT GAA GTA Lys Trp Glu Ala Pro Ser Ala Lys Lys Thr Glu Gly Ser Arg Glu Val 1400 1405 1410	5181
AAA CGG ATC GGA GAC GGT CTT TTC GTT ACG ATC GAA CCT GCA AAC GAT Lys Arg Ile Gly Asp Gly Leu Phe Val Thr Ile Glu Pro Ala Asn Asp 1415 1420 1425	5229
GTA CGT GCC AAC GAA GCC AAG GTT GTG CTC GCA GCA GAC AAC GTA TGG Val Arg Ala Asn Glu Ala Lys Val Val Leu Ala Ala Asp Asn Val Trp 1430 1435 1440	5277
GGA GAC AAT ACG GGT TAC CAG TTC TTG TTG GAT GCC GAT CAC AAT ACA Gly Asp Asn Thr Gly Tyr Gln Phe Leu Leu Asp Ala Asp His Asn Thr 1445 1450 1455	5325
TTC GGA AGT GTC ATT CCG GCA ACC GGT CCT CTC TTT ACC GGA ACA GCT Phe Gly Ser Val Ile Pro Ala Thr Gly Pro Leu Phe Thr Gly Thr Ala 1460 1465 1470 1475	5373
TCT TCC AAT CTT TAC AGT GCG AAC TTC GAG TAT TTG ATC CCG GCC AAT Ser Ser Asn Leu Tyr Ser Ala Asn Phe Glu Tyr Leu Ile Pro Ala Asn 1480 1485 1490	5421
GCC GAT CCT GTT GTT ACT ACA CAG AAT ATT ATC GTT ACA GGA CAG GGT Ala Asp Pro Val Val Thr Thr Gln Asn Ile Ile Val Thr Gly Gln Gly 1495 1500 1505	5469
GAA GTT GTA ATC CCC GGT GGT GTT TAC GAC TAT TGC ATT ACG AAC CCG Glu Val Val Ile Pro Gly Gly Val Tyr Asp Tyr Cys Ile Thr Asn Pro 1510 1515 1520	5517
GAA CCT GCA TCC GGA AAG ATG TGG ATC GCA GGA GAT GGA GGC AAC CAG Glu Pro Ala Ser Gly Lys Met Trp Ile Ala Gly Asp Gly Gly Asn Gln 1525 1530 1535	5565
CCT GCA CGT TAT GAC GAT TTC ACA TTC GAA GCA GGC AAG AAG TAC ACC Pro Ala Arg Tyr Asp Asp Phe Thr Phe Glu Ala Gly Lys Lys Tyr Thr 1540 1545 1550 1555	5613
TTC ACG ATG CGT CGC GCC GGA ATG GGA GAT GGA ACT GAT ATG GAA GTC Phe Thr Met Arg Arg Ala Gly Met Gly Asp Gly Thr Asp Met Glu Val 1560 1565 1570	5661
GAA GAC GAT TCA CCT GCA AGC TAT ACC TAT ACA GTC TAT CGT GAC GGC Glu Asp Asp Ser Pro Ala Ser Tyr Thr Tyr Thr Val Tyr Arg Asp Gly 1575 1580 1585	5709
ACG AAG ATC AAG GAA GGT CTG ACC GAA ACG ACC TAC CGC GAT GCA GGA Thr Lys Ile Lys Glu Gly Leu Thr Glu Thr Thr Tyr Arg Asp Ala Gly 1590 1595 1600	5757
ATG AGT GCA CAA TCT CAT GAG TAT TGC GTA GAG GTT AAG TAC GCA GCC Met Ser Ala Gln Ser His Glu Tyr Cys Val Glu Val Lys Tyr Ala Ala 1605 1610 1615	5805
GGC GTA TCT CCG AAG GTT TGT GTG GAT TAT ATT CCT GAC GGA GTG GCA Gly Val Ser Pro Lys Val Cys Val Asp Tyr Ile Pro Asp Gly Val Ala 1620 1625 1630 1635	5853
GAC GTA ACG GCT CAG AAG CCT TAC ACC CTG ACA GTT GTT GGA AAG ACG Asp Val Thr Ala Gln Lys Pro Tyr Thr Leu Thr Val Val Gly Lys Thr 1640 1645 1650	5901

77

ATC ACG GTA ACT TGC CAA GGC GAA GCT ATG ATC TAC GAC ATG AAC GGT Ile Thr Val Thr Cys Gln Gly Glu Ala Met Ile Tyr Asp Met Asn Gly 1655 1660 1665	5949
CGT CGT CTG GCA GCC GGT CGC AAC ACA GTT GTT TAC ACG GCT CAG GGC Arg Arg Leu Ala Ala Gly Arg Asn Thr Val Val Tyr Thr Ala Gln Gly 1670 1675 1680	5997
GGC TAC TAT GCA GTC ATG GTT GTC GTT GAC GGC AAG TCT TAC GTA GAG Gly Tyr Tyr Ala Val Met Val Val Val Asp Gly Lys Ser Tyr Val Glu 1685 1690 1695	6045
AAA CTC GCT GTA AAG TAATTCTGTC TTGGACTCGG AGACTTTGTG CAGACACTTT Lys Leu Ala Val Lys 1700 170	6100
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GTCTGGGCGA CTTTCGTTTTT ATGCCTATTA TTCTAATATA CTTCTGAAAC AATTTGTTC	6220
AAAAAGTTGC ATGAAAAGAT TATCTTACTA TCTTTGCACT GCAAAAGGGG AGTTTCCTAA	6280
GGTTTTCCCC GGAGTAGTAC GGTAATAACG GTGTGGTAGT TCAGCTGGTT AGAATACCTG	6340
CCTGTCACGC AGGGGGTCGC GGGTTCGAGT CCCGTCCATA CCGCTAAATA GCTGAAAGAT	6400
AGGCTATAGG TCATCTGAAG CAATTTTAGA AACGAATCCA AAAGCGTCTT AATCCAACG	6460
AATTAAGGCG CTTTTTCTTT GTCGCCACCC CACACGTCGG ATGAGGTTTCG GAATAGGCGT	6520
ATATTCCGTA AATATGCCTC CGGTGGTTCC ATTTTGGTTA CAAAAACAA AGGGGCTGAA	6580
AATTGTAACC ACAGACGACG TTAAGACGAT GTTTAGACGA TTGACAAATT ACTCTGTTTC	6640
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CTGTTAATCC CATACAATGG GATTCAGAAA AAGAGAAAGT CAAAGGACAT AGTGCAGAAG	6880
CACTTGAAGT CAATCGAAAG ATCGAAGAAA TCAGGGCTGA TATTCTGACC ATTTACAAAC	6940
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CCCAGGTGGG AATCAATAAA ACGGCTGCCA CTTTACAAA ATACGAAAAC AGCAAACGGC	7120
ATTTTACCCG ATTCCTCAAA GCGAAGTACA ACAGAACGGA TCTCAAATTC TCAGAGCTTA	7180
CCCCGTTGGT CATTCATAAC TTTGAGATAT ATCTGCTGAC TGTAGCCCAT TGTTGCCCGA	7240
ATACGGCAAC CAAAATCTTG AAGCTT	7266

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1704 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

78

Met Lys Asn Leu Asn Lys Phe Val Ser Ile Ala Leu Cys Ser Ser Leu
 1 5 10 15
 Leu Gly Gly Met Ala Phe Ala Gln Gln Thr Glu Leu Gly Arg Asn Pro
 20 25 30
 Asn Val Arg Leu Leu Glu Ser Thr Gln Gln Ser Val Thr Lys Val Gln
 35 40 45
 Phe Arg Met Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly
 50 55 60
 Ile Gly Gln Val Pro Thr Tyr Thr Glu Gly Val Asn Leu Ser Glu Lys
 65 70 75 80
 Gly Met Pro Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp
 85 90 95
 Thr Arg Glu Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys
 100 105 110
 Lys Asn Val Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu
 115 120 125
 Asp Pro Lys Lys Ile Pro Tyr Val Tyr Gly Lys Ser Tyr Ser Gln Asn
 130 135 140
 Lys Phe Phe Pro Gly Glu Ile Ala Thr Leu Asp Asp Pro Phe Ile Leu
 145 150 155 160
 Arg Asp Val Arg Gly Gln Val Val Asn Phe Ala Pro Leu Gln Tyr Asn
 165 170 175
 Pro Val Thr Lys Thr Leu Arg Ile Tyr Thr Glu Ile Thr Val Ala Val
 180 185 190
 Ser Glu Thr Ser Glu Gln Gly Lys Asn Ile Leu Asn Lys Lys Gly Thr
 195 200 205
 Phe Ala Gly Phe Glu Asp Thr Tyr Lys Arg Met Phe Met Asn Tyr Glu
 210 215 220
 Pro Gly Arg Tyr Thr Pro Val Glu Glu Lys Gln Asn Gly Arg Met Ile
 225 230 235 240
 Val Ile Val Ala Lys Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp
 245 250 255
 Trp Lys Asn Gln Arg Gly Leu Arg Thr Glu Val Lys Val Ala Glu Asp
 260 265 270
 Ile Ala Ser Pro Val Thr Ala Asn Ala Ile Gln Gln Phe Val Lys Gln
 275 280 285
 Glu Tyr Glu Lys Glu Gly Asn Asp Leu Thr Tyr Val Leu Leu Val Gly
 290 295 300
 Asp His Lys Asp Ile Pro Ala Lys Ile Thr Pro Gly Ile Lys Ser Asp
 305 310 315 320
 Gln Val Tyr Gly Gln Ile Val Gly Asn Asp His Tyr Asn Glu Val Phe
 325 330 335
 Ile Gly Arg Phe Ser Cys Glu Ser Lys Glu Asp Leu Lys Thr Gln Ile
 340 345 350

Asp Arg Thr Ile His Tyr Glu Arg Asn Ile Thr Thr Glu Asp Lys Trp
 355 360 365
 Leu Gly Gln Ala Leu Cys Ile Ala Ser Ala Glu Gly Gly Pro Ser Ala
 370 375 380
 Asp Asn Gly Glu Ser Asp Ile Gln His Glu Asn Val Ile Ala Asn Leu
 385 390 395 400
 Leu Thr Gln Tyr Gly Tyr Thr Lys Ile Ile Lys Cys Tyr Asp Pro Gly
 405 410 415
 Val Thr Pro Lys Asn Ile Ile Asp Ala Phe Asn Gly Gly Ile Ser Leu
 420 425 430
 Val Asn Tyr Thr Gly His Gly Ser Glu Thr Ala Trp Gly Thr Ser His
 435 440 445
 Phe Gly Thr Thr His Val Lys Gln Leu Thr Asn Ser Asn Gln Leu Pro
 450 455 460
 Phe Ile Phe Asp Val Ala Cys Val Asn Gly Asp Phe Leu Phe Ser Met
 465 470 475 480
 Pro Cys Phe Ala Glu Ala Leu Met Arg Ala Gln Lys Asp Gly Lys Pro
 485 490 495
 Thr Gly Thr Val Ala Ile Ile Ala Ser Thr Ile Asn Gln Ser Trp Ala
 500 505 510
 Ser Pro Met Arg Gly Gln Asp Glu Met Asn Glu Ile Leu Cys Glu Lys
 515 520 525
 His Pro Asn Asn Ile Lys Arg Thr Phe Gly Gly Val Thr Met Asn Gly
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 Val Pro Thr Lys Met Gln Val Thr Ala Pro Ala Gln Ile Asn Leu Thr
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 Thr Ala Thr Ile Asn Leu Thr Gly Leu Thr Asn Glu Ser Thr Leu Thr
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 Thr Asn Gly Glu Pro Asn Pro Tyr Gln Pro Val Ser Asn Leu Thr Ala
 660 665 670
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 675 680 685
 Lys Thr Asn Ala Thr Thr Asn Thr Ala Arg Ser Val Asp Gly Ile Arg
 690 695 700

80

Glu Leu Val Leu Leu Ser Val Ser Asp Ala Pro Glu Leu Leu Arg Ser
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 Gly Gln Ala Glu Ile Val Leu Glu Ala His Asp Val Trp Asn Asp Gly
 725 730 735
 Ser Gly Tyr Gln Ile Leu Leu Asp Ala Asp His Asp Gln Tyr Gly Gln
 740 745 750
 Val Ile Pro Ser Asp Thr His Thr Leu Trp Pro Asn Cys Ser Val Pro
 755 760 765
 Ala Asn Leu Phe Ala Pro Phe Glu Tyr Thr Val Pro Glu Asn Ala Asp
 770 775 780
 Pro Ser Cys Ser Pro Thr Asn Met Ile Met Asp Gly Thr Ala Ser Val
 785 790 795 800
 Asn Ile Pro Ala Gly Thr Tyr Asp Phe Ala Ile Ala Ala Pro Gln Ala
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 Asn Ala Lys Ile Trp Ile Ala Gly Gln Gly Pro Thr Lys Glu Asp Asp
 820 825 830
 Tyr Val Phe Glu Ala Gly Lys Lys Tyr His Phe Leu Met Lys Lys Met
 835 840 845
 Gly Ser Gly Asp Gly Thr Glu Leu Thr Ile Ser Glu Gly Gly Gly Ser
 850 855 860
 Asp Tyr Thr Tyr Thr Val Tyr Arg Asp Gly Thr Lys Ile Lys Glu Gly
 865 870 875 880
 Leu Thr Ala Thr Thr Phe Glu Glu Asp Gly Val Ala Thr Gly Asn His
 885 890 895
 Glu Tyr Cys Val Glu Val Lys Tyr Thr Ala Gly Val Ser Pro Lys Val
 900 905 910
 Cys Lys Asp Val Thr Val Glu Gly Ser Asn Glu Phe Ala Pro Val Gln
 915 920 925
 Asn Leu Thr Gly Ser Ala Val Gly Gln Lys Val Thr Leu Lys Trp Asp
 930 935 940
 Ala Pro Asn Gly Thr Pro Asn Pro Asn Pro Asn Pro Asn Pro Asn Pro
 945 950 955 960
 Gly Thr Thr Thr Leu Ser Glu Ser Phe Glu Asn Gly Ile Pro Ala Ser
 965 970 975
 Trp Lys Thr Ile Asp Ala Asp Gly Asp Gly His Gly Trp Lys Pro Gly
 980 985 990
 Asn Ala Pro Gly Ile Ala Gly Tyr Asn Ser Asn Gly Cys Val Tyr Ser
 995 1000 1005
 Glu Ser Phe Gly Leu Gly Gly Ile Gly Val Leu Thr Pro Asp Asn Tyr
 1010 1015 1020
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 1025 1030 1035 1040
 Trp Val Cys Ala Gln Asp Ala Asn Tyr Ala Ser Glu His Tyr Ala Val
 1045 1050 1055

Tyr Ala Ser Ser Thr Gly Asn Asp Ala Ser Asn Phe Thr Asn Ala Leu
 1060 1065 1070
 Leu Glu Glu Thr Ile Thr Ala Lys Gly Val Arg Ser Pro Glu Ala Ile
 1075 1080 1085
 Arg Gly Arg Ile Gln Gly Thr Trp Arg Gln Lys Thr Val Asp Leu Pro
 1090 1095 1100
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 1105 1110 1115 1120
 Phe Tyr Ile Asp Leu Asp Glu Val Glu Ile Lys Ala Asn Gly Lys Arg
 1125 1130 1135
 Ala Asp Phe Thr Glu Thr Phe Glu Ser Ser Thr His Gly Glu Ala Pro
 1140 1145 1150
 Ala Glu Trp Thr Thr Ile Asp Ala Asp Gly Asp Gly Gln Gly Trp Leu
 1155 1160 1165
 Cys Leu Ser Ser Gly Gln Leu Asp Trp Leu Thr Ala His Gly Gly Thr
 1170 1175 1180
 Asn Val Val Ala Ser Phe Ser Trp Asn Gly Met Ala Leu Asn Pro Asp
 1185 1190 1195 1200
 Asn Tyr Leu Ile Ser Lys Asp Val Thr Gly Ala Thr Lys Val Lys Tyr
 1205 1210 1215
 Tyr Tyr Ala Val Asn Asp Gly Phe Pro Gly Asp His Tyr Ala Val Met
 1220 1225 1230
 Ile Ser Lys Thr Gly Thr Asn Ala Gly Asp Phe Thr Val Val Phe Glu
 1235 1240 1245
 Glu Thr Pro Asn Gly Ile Asn Lys Gly Gly Ala Arg Phe Gly Leu Ser
 1250 1255 1260
 Thr Glu Ala Asn Gly Ala Lys Pro Gln Ser Val Trp Ile Glu Arg Thr
 1265 1270 1275 1280
 Val Asp Leu Pro Ala Gly Thr Lys Tyr Val Ala Phe Arg His Tyr Asn
 1285 1290 1295
 Cys Ser Asp Leu Asn Tyr Ile Leu Leu Asp Asp Ile Gln Phe Thr Met
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 1315 1320 1325
 Gly Thr Lys Ile Lys Glu Gly Leu Thr Glu Thr Thr Phe Glu Glu Asp
 1330 1335 1340
 Gly Val Ala Thr Gly Asn His Glu Tyr Cys Val Glu Val Lys Tyr Thr
 1345 1350 1355 1360
 Ala Gly Val Ser Pro Lys Glu Cys Val Asn Val Thr Ile Asn Pro Thr
 1365 1370 1375
 Gln Phe Asn Pro Val Lys Asn Leu Lys Ala Gln Pro Asp Gly Gly Asp
 1380 1385 1390
 Val Val Leu Lys Trp Glu Ala Pro Ser Ala Lys Lys Thr Glu Gly Ser
 1395 1400 1405

Arg Glu Val Lys Arg Ile Gly Asp Gly Leu Phe Val Thr Ile Glu Pro
1410 1415 1420

Ala Asn Asp Val Arg Ala Asn Glu Ala Lys Val Val Leu Ala Ala Asp
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Asn Val Trp Gly Asp Asn Thr Gly Tyr Gln Phe Leu Leu Asp Ala Asp
1445 1450 1455

His Asn Thr Phe Gly Ser Val Ile Pro Ala Thr Gly Pro Leu Phe Thr
1460 1465 1470

Gly Thr Ala Ser Ser Asn Leu Tyr Ser Ala Asn Phe Glu Tyr Leu Ile
1475 1480 1485

Pro Ala Asn Ala Asp Pro Val Val Thr Thr Gln Asn Ile Ile Val Thr
1490 1495 1500

Gly Gln Gly Glu Val Val Ile Pro Gly Gly Val Tyr Asp Tyr Cys Ile
1505 1510 1515 1520

Thr Asn Pro Glu Pro Ala Ser Gly Lys Met Trp Ile Ala Gly Asp Gly
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Gly Asn Gln Pro Ala Arg Tyr Asp Asp Phe Thr Phe Glu Ala Gly Lys
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Lys Tyr Thr Phe Thr Met Arg Arg Ala Gly Met Gly Asp Gly Thr Asp
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Met Glu Val Glu Asp Asp Ser Pro Ala Ser Tyr Thr Tyr Thr Val Tyr
1570 1575 1580

Arg Asp Gly Thr Lys Ile Lys Glu Gly Leu Thr Glu Thr Thr Tyr Arg
1585 1590 1595 1600

Asp Ala Gly Met Ser Ala Gln Ser His Glu Tyr Cys Val Glu Val Lys
1605 1610 1615

Tyr Ala Ala Gly Val Ser Pro Lys Val Cys Val Asp Tyr Ile Pro Asp
1620 1625 1630

Gly Val Ala Asp Val Thr Ala Gln Lys Pro Tyr Thr Leu Thr Val Val
1635 1640 1645

Gly Lys Thr Ile Thr Val Thr Cys Gln Gly Glu Ala Met Ile Tyr Asp
1650 1655 1660

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Ala Gln Gly Gly Tyr Tyr Ala Val Met Val Val Val Asp Gly Lys Ser
1685 1690 1695

Tyr Val Glu Lys Leu Ala Val Lys
1700

WE CLAIM:

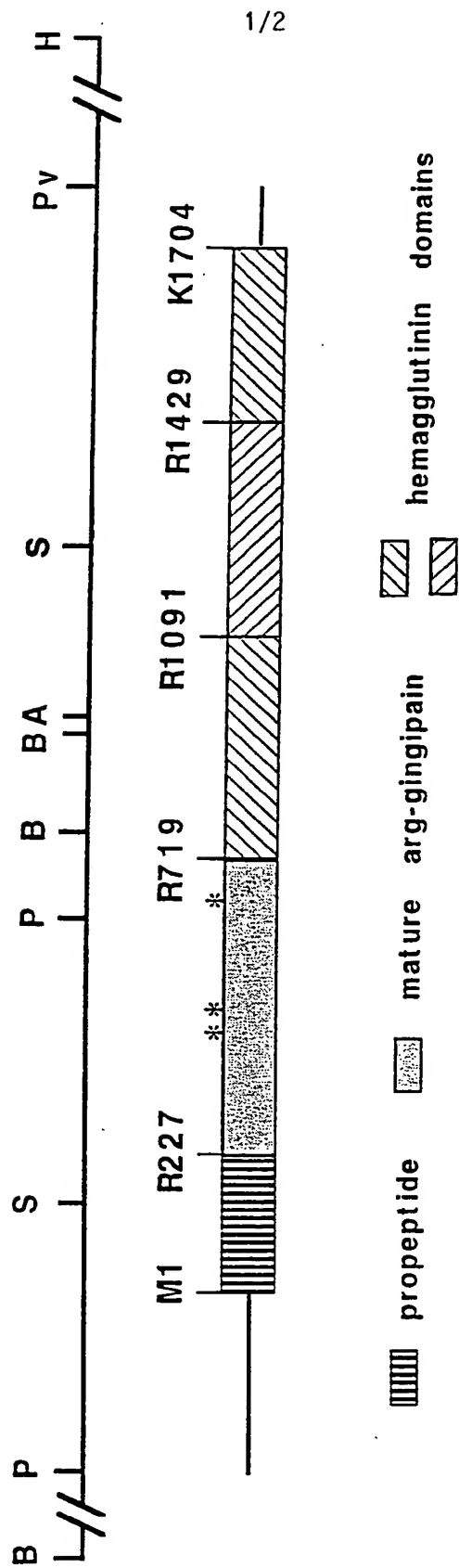
- 1
1 1. A recombinant DNA molecule comprising a nucleotide sequence
2 encoding an Arg-gingipain protein having an amino acid
3 sequence selected from group consisting of sequences as
4 given in SEQ ID NO:4 from amino acid 1 through amino acid
5 510, SEQ ID NO:11 from amino acid 228 through amino acid
6 719, and an amino acid sequence having at least about 85%
7 amino acid sequence identity with a sequence as given in
8 SEQ ID NO:11 from amino acid 228 to amino acid 719.
- 1 2. The recombinant DNA molecule of claim 1, wherein said
2 nucleotide sequence is as given in one of SEQ ID NO:3 from
3 nucleotide 1630 through nucleotide 3105 and SEQ ID NO:10
4 from nucleotide 1630 through nucleotide 3105.
- 1 3. A recombinant DNA molecule comprising a nucleic acid
2 portion encoding a high molecular weight Arg-gingipain
3 comprising an enzymatically active protease component and
4 a hemagglutinin component.
- 1 4. The recombinant DNA molecule of claim 3 wherein said
2 encoded high molecular weight Arg-gingipain has an
3 enzymatically active protease component having an amino
4 acid sequence as given in one of SEQ ID NO:4 from amino
5 acid 1 to amino acid 510 and SEQ ID NO:11 from amino acid
6 228 to amino acid 719.
- 1 5. The recombinant DNA molecule of claim 4 herein said high
2 molecular weight Arg-gingipain has an enzymatically active
3 protease component having an amino acid sequence as given
4 in SEQ ID NO:11 from amino acid 228 to amino acid 719 and
5 a hemagglutinin component having an amino acid sequence
6 selected from the group consisting from amino acid 720 to
7 amino acid 1091, from amino acid 1092 to amino acid 1429
8 and from amino acid 1430-1704, each as given in SEQ ID NO:11.

- 1 6. The recombinant DNA molecule of claim 4 wherein said mature
2 enzymatically active protease component is encoded by a
3 nucleotide sequence as given in one of SEQ ID NO:3 from
4 nucleotide 1630 to nucleotide 3105, in SEQ ID NO:10 from
5 nucleotide 1630 to nucleotide 3105 or a nucleotide sequence
6 having at least 70% homology to one of said sequences.

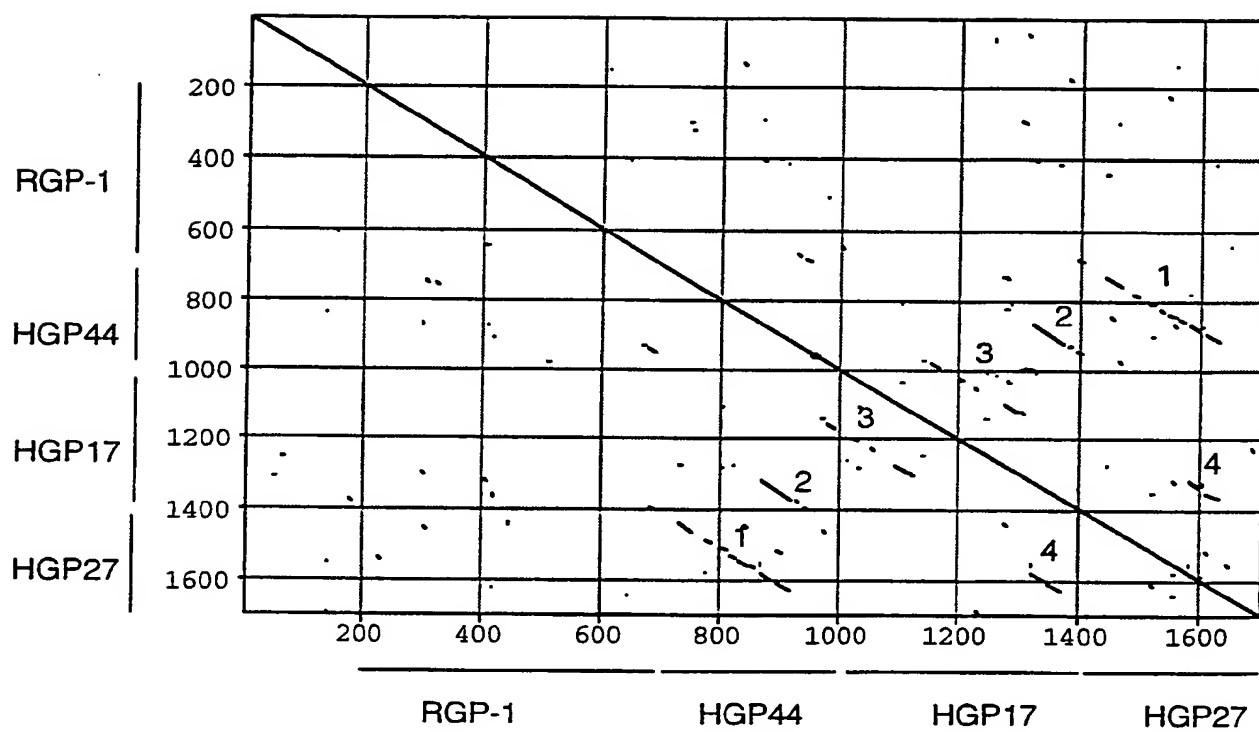
- 1 7. The recombinant DNA molecule of claim 1 wherein said Arg-
2 gingipain is encoded within a nucleotide sequence as given
3 in SEQ ID NO:10 from nucleotide 949-6063, or a nucleotide
4 sequence having at least about 70% sequence homology
5 thereto.

- 1 8. The recombinant DNA molecule of claim 7 wherein said Arg-
2 gingipain is expressed as a prepolyprotein having an amino
3 acid sequence as given in SEQ ID NO:11.

- 1 9. The recombinant DNA molecule of claim 8 wherein the
2 nucleotide sequence encoding said polyprotein is as given
3 in SEQ ID NO:10 from nucleotide 949 to nucleotide 6063.



2/2



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/10283

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 435/69.1, 195, 212, 213, 220, 320.1; 536/22.1, 23.1, 23.2, 23.7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 195, 212, 213, 220, 320.1; 536/22.1, 23.1, 23.2, 23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 267, No. 26, issued 15 September 1992, Chen et al., "Purification and Characterization of a 50-kD Cysteine Proteinase (Gingipain) from <i>Porphyromonas gingivalis</i> ", pages 18896-18901, see entire document.	1-9
Y	FASEB JOURNAL ABSTRACT, Vol. 5, No. 4, issued 1991, Potempa et al., "Purification and Characterization of a 50 kDa Cysteine Proteinase of <i>Porphyromonas gingivalis</i> ", page A829, abstract no. 2667, see entire abstract.	1-9
Y	SCIENCE, Vol. 239, issued 11 March 1988, Lee et al., "Generation of cDNA Probes Directed by Amino Acid Sequence: Cloning of Urate Oxidase", pages 1288-1291, see entire document.	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

^a	Special categories of cited documents:	^T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
^A	document defining the general state of the art which is not considered to be of particular relevance	^X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
^E	earlier document published on or after the international filing date	^Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
^L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	^Z	document member of the same patent family
^O	document referring to an oral disclosure, use, exhibition or other means		
^P	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

18 DECEMBER 1994

Date of mailing of the international search report

05 JAN 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/10283

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 17/00, 19/00, 21/00; C12N 9/14, 9/48, 9/52, 9/76, 15/00; C12P 21/06

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, STN, BIOSIS

search terms: bacteroides gingivalis, porphyromonas gingivalis, gingipain, arg gingipain, proteinase?, arg, gingipain-1, gingipain-2